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Potential effects of mixed infections in ticks on transmission dynamics of pathogens: comparative analysis of published records

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Abstract Ticks are often infected with more than one pathogen, and several field surveys have documented nonrandom levels of coinfection. Levels of coinfection by pathogens in four tick species were analyzed using published infection data. Coinfection patterns of pathogens in field-collected ticks include numerous cases of higher or lower levels of coinfection than would be expected due to chance alone, but the vast majority of these cases can be explained on the basis of vertebrate host associations of the pathogens, without invoking interactions between pathogens within ticks. Nevertheless, some studies have demonstrated antagonistic interactions, and some have suggested potential mutualisms, between pathogens in ticks. Negative or positive interactions between pathogens within ticks can affect pathogen prevalence, and thus transmission patterns. Probabilistic projections suggest that the effect on transmission depends on initial conditions. When the number of tick bites is relatively low (e.g., for ticks biting humans) changes in prevalence in ticks are predicted to have a commensurate effects on pathogen transmission. In contrast, when the number of tick bites is high (e.g., for wild animal hosts) changes in pathogen prevalence in ticks have relatively little effect on levels of transmission to reservoir hosts, and thus on natural transmission cycles.

Keywords Mixed infections · Ticks · Coinfection · Transmission dynamics

Introduction

Ticks serve as vectors for numerous pathogens, and individual ticks are often infected with more than one pathogenic organism. Multiple infections can have medical significance, because coinfection can increase severity of symptoms in humans and animals (Belongia 2002; Thomas et al. 2001). Mixed infections in ticks can also potentially influence transmission dynamics, because of either interactions between the pathogens within the

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ticks, or pathogenic effects on tick behavior or survival. This applies both to vertebrate pathogens in ticks and to entompathogenic organisms. For example, Ross and Levin (2004) found that some strains of *Anaplasma phagocytophilum*, the etiologic agent of granulocytic anaplasmosis in humans, affect molting of *Ixodes scapularis* ticks. Hornbostel et al. (2004) found sublethal effects of the entomopathogenic fungus *Metarhizium anisopliae* on fecundity and body mass of *I. scapularis*. In cases of mixed infections, these pathogens could potentially influence transmission of other pathogens in the tick by virtue of their effects on tick behavior and survival.

Investigators have used two major approaches to studying the ecological features of mixed infections in ticks. One is to infect ticks with single or multiple pathogens in the laboratory, and to quantify differences in pathogen persistence and transmission to lab animals. This approach gives direct information about pathogen interactions, but these interactions might differ in the laboratory than under field conditions. The second approach is to quantify infection with each pathogen in field collected ticks, and to test whether the prevalence of mixed infections is higher or lower than would be expected on the basis of chance alone. This approach provides field tests of the interactions of pathogens, but interpretation can be difficult because the number of mixed infections results from factors other than just interactions of pathogens. For example, if two pathogens occur in different vertebrate host species, then these pathogens will generally not be found together in nymphal ticks, even if they have no interaction within the tick. Even for ticks with broad host ranges, nymphs have generally fed only once (as larvae) and have therefore acquired pathogens from only one vertebrate host species. In this case, proportions of mixed infections in nymphs would be expected to be lower than in adult ticks, because the nymphs have only fed once while adults have fed twice, and the adults might have picked up infections from different host species.

To fully assess the causes of observed levels of coinfection at a given site, it is necessary to conduct laboratory studies and in-depth local field studies. However, it is also worthwhile to ask whether broad patterns of coinfection from numerous field sites fit the hypotheses of antagonistic or of mutualistic interactions within ticks, or whether tick host associations are adequate to explain the observed patterns. I briefly review some relevant research below. A comparative analysis of published data from numerous field sites follows.

Evidence of interactions between pathogens within tick hosts

Negative relationships between pathogens

A well-known example of negative interactions of rickettsiae within ticks is the transovarial transmission interference of *Rickettsia rickettsii* (agent of Rocky Mountain Spotted Fever) in *Dermacentor andersoni* ticks coinfected with the nonpathogenic Spotted Fever Group rickettsia *R. peacockii* (Burgdorfer et al. 1981; Macaluso and Azad 2005). Ticks coinfected with both rickettsiae vertically transmit only the nonpathogenic species, which influences the distribution of *R. rickettsii*. Similar negative interactions apparently occur among other arthropod-transmitted rickettsiae (Macaluso et al. 2002; Rudakov et al. 2003).

De la Fuente et al. (2002) inoculated *I. scapularis* cells with different strains of *Anaplasma marginale*, and found that only one strain persisted. Furthermore, *A. ovis* infection excluded infection by *A. marginale* in *I. scapularis* cells. When *Dermacentor variabilis*

males fed sequentially on calves infected with different strains of *A. marginale*, only one strain persisted in the ticks (De la Fuente et al. 2003).

Negative interactions in ticks have also been reported for pathogens other than rickettsia. For example, Alekseev et al. (1996) presented evidence that *Borrelia* infection suppressed replication of tick-borne encephalitis virus in *Ixodes persulcatus*. Mather et al. (1987) found that *I. scapularis* that were parasitized by the encyrtid wasp *Ixodiphagus hookeri* were not infected with *Borrelia burgdorferi* and were rarely infected with *Babesia microti*, two pathogens that were common in ticks not parasitized by wasp larvae. However, this phenomenon could have resulted from the host-finding behavior of the wasp (rather than from pathogen interactions within ticks), because *I. hookeri* might have preferentially parasitized ticks attached to white-tailed deer, which is a poor reservoir for both pathogens (Samish et al. 2004).

Positive relationships between pathogens

Sutáková and Rehácek (1990) found increased spread of *Coxiella burnetii* into tissues of *Dermacentor reticulatus* in the presence of *Rickettsia phytoseiuli*. In a survey of 738 *Ixodes persulcatus* ticks in Russia for infection with *Babesia microti* (Alekseev et al. 2003), all 7 infected ticks were also infected with other pathogens (including *Borrelia* spp. and tick-borne encephalitis virus (TBEV)). Postic et al. (1997) found high prevalence of mixed infections of *Borrelia* genospecies in ticks and hosts, also in Russia. In the United States, Mixson et al. (2006) found higher than random levels of coinfection of *Ehrlichia chafeensis* and *E. ewingi* in *Amblyomma americanum* ticks. *Rickettsia amblyommii* and *Borrelia lonestari* also showed higher than random levels of association in this tick. Of course, these high levels of coinfection in field-collected ticks might have resulted from ecological factors relating to pathogen infections in reservoir hosts and tick feeding preferences, rather than from mutualistic interactions of pathogens within the ticks.

No interactions between pathogens

Levin and Fish (2000) studied transmission of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* by *Ixodes scapularis* to white-footed mice in the laboratory. They found no differences in transmission rates between singly infected and coinfected ticks. In field studies, Korenberg et al. (1999) found no evidence of interference between TBEV and *Borrelia burgdorferi* s.l. in *Ixodes persulcatus* ticks in Russia, and Morozova et al. (2002) found these pathogens to be distributed independently in *I. persulcatus* in western Siberia. Hornbostel et al. (2005) found no differences in prevalence of *B. burgdorferi* s.s. in *Ixodes scapularis* ticks regardless of whether or not they were infected with the entomopathogenic fungus *Metarhizium anisopliae*. Swanson and Norris (2007) found that *Borrelia burgdorferi* s.s. and *Rickettsia* spp. were distributed independently in *I. scapularis* ticks. They found that some other pathogens co-occurred with *B. burgdorferi* more frequently than expected due to chance alone, but they attributed these cases to shared enzootic cycles rather than to interactions within the ticks.

These examples provide evidence that some pathogens display antagonistic interactions in ticks, others display mutualisms, and many apparently do not interact within the ticks. However, they do not provide insight into the frequency of each type of interaction among pathogens within ticks. In the following sections I assess the frequencies of various types of interactions between pathogens within ticks by compiling data from several field studies

that measured infections of various pathogens within ticks at various sites, and testing whether the proportion of coinfections was significantly higher or lower than would be expected due to chance alone. I then consider the implications of coinfections for transmission dynamics of these pathogens.

Methods

The scientific literature was surveyed to find studies that reported raw data on infection rates of pathogens in ticks, including mixed infections, with sample sizes large enough for statistical analysis. This survey was restricted to papers where the pathogen strains were identified (thus mostly to recent literature) and where the numbers of individuals infected singly with each pathogen, the number coinfected with both, and the number not infected with either, could be ascertained. Much of this work has been restricted to pathogens of public health importance, so mostly pathogens that have been at least tentatively implicated in human illness were included. The numbers of ticks positive and negative for each pathogen at each site were compiled in 2×2 contingency tables, and simple chi-square tests (SAS, version 9.2) was used to assess significance. To quantify the degree of departure of the number of mixed infections from independence, I developed an index of coinfection (I_c), defined as the difference of the number of coinfections from the number expected due to chance alone, as a percentage of the total number of infected ticks in the sample. If a = number of ticks infected with both pathogens, b = number infected only with pathogen 1, c = number infected only with pathogen 2, and d = number not infected with either pathogen, then the number of observed coinfections (O) equals a, the expected number of coinfected ticks due to chance alone (E) is given by: $E = ((a + b)(a + c))/(a + b + c + d)$, and the total number of ticks infected by either or both pathogens (N) is: $N = a + b + c$. The index of coinfection (I_c) is:

$$I_c = ((O - E)/N) \times 100$$

Note that I_c is positive when the number of coinfections is greater than expected, and negative when there are fewer coinfections than would be expected due to chance alone.

To assess the potential implications of coinfection for pathogen transmission dynamics, I used a simple binomial model of the probability of exposure to a pathogen (P_e) when an animal is bitten by n ticks, and with a prevalence of infection in ticks of k_v (Ginsberg 1993, 2001). P_e is the probability that at least one of the n ticks is infected:

$$P_e = 1 - (1 - k_v)^n$$

Results

Levels of coinfection of various pathogens in *Ixodes ricinus* and *I. persulcatus* are shown in Table 1. The number of mixed infections differed signficantly from the expectation due to chance alone in about half the cases. I_c was positive (when significant) for mixed infections of *B. burgdorferi s.s.* and *B. afzelii*, of *B. garinii* and *B. valaisiana*, of *B. burgdorferi s.s.* and *B. garinii*, and of *B. burgdorferi* s.l. and *Anaplasma phagocytophilum*. I_c was negative (when significant) for interactions between *B. afzelii* and *B. valaisiana*. I_c was sometimes positive and sometimes negative for coinfections between *B. afzelii* and *B. garinii*.

Table 1 Patterns of coinfection between pathogen species in *Ixodes ricinus* and in *I. persulcatus* ticks. P = probability from chi-square test, I_c = index of coinfection

Tick species	Pathogen 1	Pathogen 2	Stage(s)	Number sampled	Number coinfected	Total number infected	P	I_c	Reference
Ixodes ricinus	*Borrelia burgdorferi* s.s.	*B. afzelii*	nymphs	411	3	25	<0.0001	+10.25	Kirstein et al. (1997)
			adults	90	1	26	0.478	+1.71	Golubić et al. (1998)
	B. afzelii	*B. garinii*	nymphs	411	0	30	0.516	−1.31	Kirstein et al. (1997)
			nymphs	198	1	30	0.878	−0.51	Hanincová et al. (2003b)
			adults	90	0	28	0.275	−2.98	Golubić et al. (1998)
			adults	239	0	65	0.018	−6.44	Hanincová et al. (2003b)
			adults	147	1	46	0.135	−4.92	Hanincová et al. (2003b)
			adults	154	0	35	0.229	−3.23	Hanincová et al. (2003b)
			nymphs + adults	200	10	53	0.007	+9.53	Rauter et al. (2002)
			nymphs + adults	256	26	126	0.022	+5.52	Rauter et al. (2002)
			nymphs + adults	196	8	33	<0.0001	+18.21	Rauter et al. (2002)
			nymphs + adults	200	9	74	0.507	+2.00	Rauter et al. (2002)
			nymphs + adults	203	7	45	0.001	+10.01	Rauter et al. (2002)
	B. afzelii	*B. valaisiana*	nymphs	411	2	41	0.062	+3.38	Kirstein et al. (1997)
			nymphs	130	0	28	0.393	−2.06	Hanincová et al. (2003b)
			nymphs	198	2	29	0.391	+2.93	Hanincová et al. (2003b)

Table 1 continued

Tick species	Pathogen 1	Pathogen 2	Stage(s)	Number sampled	Number coinfected	Total number infected	*P*	I_c	Reference
			nymphs	174	0	31	0.197	−4.45	Hanincová et al. (2003b)
			adults	239	0	52	0.111	−3.86	Hanincová et al. (2003b)
			adults	147	0	46	0.038	−6.63	Hanincová et al. (2003b)
			adults	154	0	67	0.0006	−10.68	Hanincová et al. (2003b)
			adults	90	4	30	0.239	+5.00	Golubić et al. (1998)
	B. garinii	*B. valaisiana*	nymphs	411	8	51	<0.0001	+11.74	Kirstein et al. (1997)
			nymphs	198	3	21	0.005	+10.82	Hanincová et al. (2003b)
			adults	90	0	12	0.557	−2.50	Golubić et al. (1998)
			adults	239	4	33	0.008	+8.32	Hanincová et al. (2003b)
			adults	147	2	27	0.596	+2.12	Hanincová et al. (2003b)
			adults	154	0	44	0.153	−3.36	Hanincová et al. (2003b)
	Borrelia burgdorferi s.l.	*Anaplasma phagocytophilum*	nymphs	114	1	16	0.260	+3.95	Hildebrandt et al. (2003)
			adults	112	15	59	0.234	+4.72	Christova et al. (2001)
			adults	424	21	112	<0.0001	+10.08	Stańczak et al. (2002)

Table 1 continued

Tick species	Pathogen 1	Pathogen 2	Stage(s)	Number sampled	Number coinfected	Total number infected	*P*	I_c	Reference
			adults	303	25	117	0.004	+7.62	Stańczak et al. (2004)
Ixodes persulcatus	*Borrelia burgdorferi* s.s.	*B. afzelii*	adults	1280	10	185	<0.0001	+4.62	Alekseev et al. (2003)
	B. burgdorferi s.s.	*B. garinii*	adults	1280	28	85	<0.0001	+30.75	Alekseev et al. (2003)
	B. afzelii	*B. garinii*	adults	1280	68	300	<0.0001	+14.76	Alekseev et al. (2003)

Mixed infections of diverse pathogens in *I. scapularis* and *A. americanum* are shown in Table 2. I_c was positive for coinfections of *Ehrlichia chafeensis* and *E. ewingi* in *A. americanum* ticks. No other significant levels of coinfection were observed.

One major effect of positive or negative interactions among pathogens within a tick would be to raise or lower infection prevalence of the affected pathogen. The potential effects on transmission dynamics are shown in Fig. 1. The effects differ when tick populations are low (e.g., one tick bite in Fig. 1) compared to when tick populations are high (e.g., 50 tick bites) because the probability of exposure to the pathogen rapidly reaches 1.0 when hosts are exposed to numerous tick bites. When tick numbers are low, the effect on P_e of changes in the proportion of ticks infected is more or less linear. When the number of tick bites is high, on the other hand, P_e is near 1.0 unless the proportion of ticks infected is near zero (Fig. 1).

Discussion

Nymphal ticks have generally fed only once, as larvae. Therefore, mixed infections in nymphs (for pathogens such as *Borrelia* spp. that are not generally passed vertically) presumably result from feeding on a host infected with both pathogens. Of course, some ticks might carry mixed infections because, for example, *Borrelia* infections are occasionally passed vertically, and larval feeding can be interrupted, which can result in a second larval feeding, but these are probably relatively rare phenomena (Nefedova et al. 2004). Only two significant positive associations of *Borrelia* spp. were found in nymphal ticks. One was for *B. burgdorferi* s.s. and *B. afzelii* in *I. ricinus*. Both of these *Borrelia* species commonly infect rodents, suggesting that these ticks fed as larvae on rodents with mixed infections. The other was for *B. valaisiana* and *B. garinii* in *I. ricinus*. These *Borrelia* species commonly infect songbirds (Hanincová et al. 2003b), and these ticks might have attached to coinfected birds. In both cases, the positive associations suggest that there are no negative interactions between these *Borrelia* species within *I. ricinus*. In fact, I found no significant negative associations between *B. burgdorferi* s.s and *B. afzelii* or between *B. valaisiana* and *B. garinii* in any stage of any tick species.

Adult ticks have fed twice, once as larvae and once as nymphs. Higher than expected occurrence of pathogens in adult ticks could result from immature ticks feeding on frequently coinfected hosts or from positive interactions among the pathogen species (e.g., higher transmission efficiency of one pathogen when the tick is already infected with the other, or higher pathogen survival in ticks in mixed infections than in single infections). Negative associations presumably result from negative interactions between the pathogens. Some *Borrelia* species primarily infect mammals while others primarily infect birds, but this would only lead to a negative association within a tick species if individual ticks tended to feed on mammals as both larvae and nymphs, while other individuals of the same species tended to feed on birds as both larvae and nymphs. This seems unlikely, although it is plausible that when engorged larvae drop from their hosts, they might be left in microhabitats that would favor them biting the same type of host after molting to the nymphal stage. This possibility is worth additional study.

Most pairs of *Borrelia* species showed at least some examples of higher than expected coinfections. Reservoirs of *B. burgdorferi* s.s. include both mammals (Levine et al. 1985; Kurtenbach et al. 2002) and birds (Richter et al. 2000; Ginsberg et al. 2005). Thus positive associations could result from ticks feeding on coinfected hosts of both mammal-associated species such as *B. afzelii* (Hu et al. 1997; Hanincová et al. 2003a), and with other *Borrelia*

Table 2 Patterns of coinfection between pathogen species in *Ixodes scapularis* and in *Amblyomma americanum* ticks. P = probability from chi-square test, I_c = index of coinfection

Tick species	Pathogen 1	Pathogen 2	Stage	Number sampled	Number coinfected	Total number infected	P	I_c	Reference
Ixodes scapularis	*Borrelia burgdorferi*	*Anaplasma phagocytophilum*	adults	147	4	79	0.715	−0.67	Schulze et al. (2005)
	Anaplasma phagocytophilum	*Babesia odocoilei*	adults	68	0	15	0.308	−5.49	Steiner et al. (2006)
Amblyomma americanum	*Ehrlichia chaffeensis*	*E. ewingi*	adults	121	5	20	0.0002	+18.80	Schulze et al. (2005)
	Borrelia lonestari	*E. chaffeensis*	adults	121	1	25	0.721	−0.67	Schulze et al. (2005)
	E. ewingi	*B. lonestari*	adults	121	0	21	0.297	−4.33	Schulze et al. (2005)

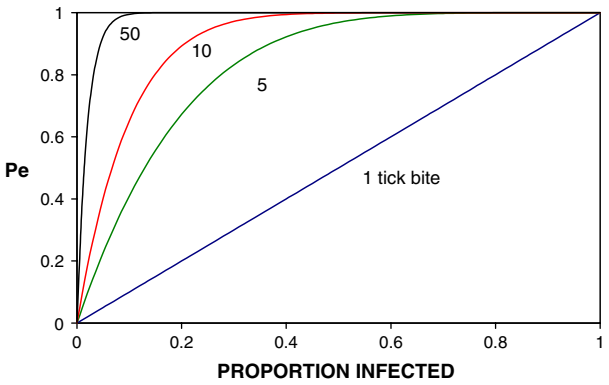

Fig. 1 Probability of host exposure to a pathogen (P_e) at different levels of tick abundance and pathogen prevalence in ticks

species associated with both mammals and birds such as *B. garinii* (Kurtenbach et al. 2002). The one example of a lower than expected level of coinfection of *B. afzelii* and *B. garinii* in *I. ricinus* (Table 1) suggests that there is no positive interaction of these pathogens within the tick.

The one pair of *Borrelia* genospecies that generally showed lower than expected levels of coinfection was *B. afzelii* with *B. valaisiana* in *I. ricinus* (Table 1). As already mentioned, *B. afzelii* primarily infects mammals while *B. valaisiana* primarily infects birds, so individual ticks feeding on coinfected hosts is unlikely. Nevertheless, nymphal ticks can attach to different hosts than larvae, so this negative association might suggest a negative interaction between these pathogens within *I. ricinus*. However, the one case in Table 1 of a marginally positive association in nymphs (data from Kirstein et al. 1997) suggests that there is no negative interaction between these pathogens within *I. ricinus* ticks. Infection and transmission experiments with these *Borrelia* species in *I. ricinus* in the lab could help clarify the mechanism responsible for these results.

The positive associations between *B. burgdorferi* s.l. and *A. phagocytophilum* (Table 1) could have resulted largely from ticks feeding on coinfected hosts because mice and other small mammals serve as reservoirs for both of these pathogens (Kurtenbach et al. 2002; Telford et al. 1996). Similarly, the positive association of *Ehrlichia chaffeensis* with *E. ewingi* in *Amblyomma americanum* ticks (Table 2) could result from the likely role of white-tailed deer, *Odocoileus virginianus*, as the primary reservoir of both rickettsial species (Dawson et al. 2005; Paddock et al. 2005).

The abundant examples of higher than expected levels of coinfection in ticks suggest that hosts are frequently infected with more than one pathogen species. This could result from positive interactions of pathogens within the vertebrate hosts, or it could simply result from large tick populations. When tick populations are large enough that individual host animals are bitten by numerous ticks, then the probability that individual host animals are exposed to more than one pathogen is high.

Implications for pathogen transmission patterns

In general, these results provide little evidence of negative interactions among pathogens within ticks (with the possible exception of *B. afzelii* and *B. valaisiana*). Nevertheless, there are a few examples in which negative interactions between pathogens have been documented, such as the interaction of *Rickettsia peacockii* with *R. rickettsii* (Macaluso

and Azad 2005), and the interactions among selected strains of *Anaplasma marginale* (de la Fuente et al. 2003). There have also been some reports of positive interactions among pathogens, such as those of *Babesia microti* with other pathogens in *I. persulcatus* (Alekseev et al. 2003). These interactions could potentially influence transmission dynamics by lowering or raising infection prevalence in ticks, and thus affecting the probability that an individual vertebrate will be exposed to the bite of an infected tick. The potential implications of negative or positive interactions among pathogens in mixed infections apparently differ for humans than for reservoir hosts involved in natural transmission cycles. Most humans are bitten by relatively few ticks per year, even in high-incidence sites for Lyme borreliosis (Ginsberg 1993). For a person who is bitten by one tick in a given year, a negative interaction among pathogens within the tick would lower the probability of exposure to the pathogen linearly with the lowering of infection prevalence in ticks (Fig. 1). If infection prevalence is lowered from 0.4 to 0.2, for example, then the probability of exposure is also lowered from 0.4 to 0.2. In contrast, for a wild reservoir host that is constantly exposed to ticks, and is bitten by 50 or more ticks per year, the probability of exposure remains 1.0, even when the prevalence of infection in ticks has been lowered from 0.4 to 0.2 (Fig. 1).

This result applies to positive interactions between pathogens within ticks as well. If prevalence of a pathogen in ticks increases from 0.4 to 0.6, the risk of human disease would increase to the same extent (for humans bitten by one tick per year), but the natural transmission cycle would not be affected. Therefore, interactions among pathogens in ticks that influence pathogen prevalence will tend to have greater direct effects on human disease incidence than on the dynamics of natural transmission cycles. This result does not apply to pathogens with low prevalence in ticks (prevalences below 0.2 in Fig. 1) where changes in prevalence have substantial effects on the probability of exposure. The prevalence level at which changes in prevalence affect transmission depends on tick abundance. For example, at sites where individual hosts are bitten by 1,000 ticks or more (e.g., deer in some locales), P_e is nearly 1.0 even at low pathogen prevalence levels in ticks.

This analysis pertains primarily to cases where transmission is primarily horizontal, such as for *Borrelia burgdorferi* s.l. In contrast, when vertical transmission contributes strongly to pathogen maintenance, as in *R. rickettsii* in *D. andersoni* (Schriefer and Azad 1994), transmission interference by other rickettsia can apparently have strong effects on prevalence.

Hornbostel et al. (2005) found no effect of infection of *I. scapularis* ticks with the entomopathogenic fungus, *Metarhizium anisopliae*, on the prevalence of *B. burgdorferi* in these ticks. Beyond this observation, however, interactions between entomopathogens and zoonotic pathogens in ticks have received little attention. Such interactions warrant further study because they could potentially influence the effectiveness of entomopathogens as biocontrol agents for vector-borne diseases.

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