

Evaluation of *Dermacentor* species naturally infected with *Rickettsia raoultii*

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INTRODUCTION

In 1999, three new spotted fever group rickettsial genotypes, i.e. RpA4, DnS12 and DnS28, were detected in ticks from Russia by PCR [1]. Rickettsiae with identical genotypes were later isolated in Russia and France, and classified within a new species named *Rickettsia raoultii* [2]. This rickettsial species is currently considered as an emerging pathogen and is an agent of tick-borne lymphadenitis [2]. *R. raoultii* has been found in several European and Asian countries, mostly in *Dermacentor* ticks (*D. nuttallii*, *D. marginatus*, *D. reticulatus*, *D. silvarum*), and also in *Rhipicephalus pumilio*, *Haemaphysalis concinna* and *Ixodes persulcatus* [1–4]. However, to date, the relationships between ticks and *R. raoultii* have not been studied.

In the present study, we investigated the efficiency of transovarial transmission and intensity of multiplication of three *R. raoultii* genotypes in four naturally-infected *Dermacentor* species. We used the method of experimental modelling of a natural cycle of the metamorphosis of vectors (MEMNCMV) that allows the study of the role of tick species as vectors of infectious agents, including rickettsiae.

MATERIALS AND METHODS

We compared the efficiency of vertical transmission and concentration of *R. raoultii* (genotypes RpA4, DnS14 and DnS28) in pre-imaginal stages of four *Dermacentor* species: *D. nuttalli*, *D. silvarum*, *D. marginatus* and *D. reticulatus*. All ticks, collected in different regions of Siberia (Omsk region and Altai (Western Siberia), Buryatiya (Eastern Siberia) and Kazakhstan), were naturally infected with *R. raoultii*, as evaluated in all evolutionary stages by *ompA* PCR and sequencing.

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Genotypic identification of studied strains was performed using *ompA* and *gltA* gene amplification and sequencing. Tick cultivation in laboratory conditions, study of transovarial and transstadial transmission rates, and study of rickettsial concentration (number of rickettsiae per visual field) in tick preimaginal stages, were carried out using immunofluorescence as previously described [5].

RESULTS

Rickettsiae naturally infecting the laboratory tick cohorts were identified as various genotypes of *R. raoultii* (RpA4 in four laboratory cohorts of *D. marginatus* and one cohort of *D. reticulatus*; DnS14 in one cohort of *D. silvarum*; DnS28 in two cohorts of *D. nuttalli* and one cohort of *D. silvarum*). Levels of transovarial transmission ranged from 43% in *D. nuttalli* infected with DnS28 (in the first generation tick, increasing to 89% in the second generation) to 100% in *D. marginatus* infected with RpA4. The level of transstadial transmission was high in all cohorts: from 82% to 100%. The level of rickettsial concentration in larvae (IF test) varied insignificantly: from 3.4 copies (*D. reticulatus* infected with RpA4) to 10 copies per visual field (*D. silvarum* infected with DnS14). In nymphs, the range of rickettsial concentration was greater: from 3.6 copies (*D. silvarum* infected with DnS28) to 49 copies per visual field (*D. marginatus* infected with RpA4) (Table 1).

DISCUSSION

The life cycles of most tick-borne rickettsiae are incompletely known. Transovarial transmission of rickettsiae in their recognised vectors has been demonstrated for several spotted fever group species, including *R. rickettsii*, *R. sibirica*, *R. africae*, *R. helvetica*, *R. parkeri* and *R. slovaca*. In some instances, transovarial transmission of a particular *Rickettsia* species may occur in a particular tick but cannot be sustained for more than one generation. When rickettsiae are transmitted efficiently both transstadially and transovarially in a

Table 1. Efficiency of transovarial (TOT) and transstadial (TST) transmission and mean of rickettsiae per visual field of different strains of *R. raoultii*

Strain name	Genotype	Species of tick	Region	TOT (%)	TST (%)	Rickettsial concentration in larvae before/after feeding	Rickettsial concentration in nymphs before/after feeding
Elanda-23/95 F1	DnS28	<i>D. nuttalli</i>	Altai	86.0%	NA	5.7/23	9.2/48.5
Elanda-23/95 F3	DnS28	<i>D. nuttalli</i>	Altai	99.5%	98.3%	6/24	10/50
Elanda-29/96 F1	DnS28	<i>D. nuttalli</i>	Altai	43.0%	86.4%	6.1/33.2	31/51
Elanda-29/96 F3	DnS28	<i>D. nuttalli</i>	Altai	90.0%	100%	6/36	39/54
Karaganda-7/98	RpA4	<i>D. marginatus</i>	Kazakhstan	99.0%	100%	7/49	46/53
Karaganda-8/98	RpA4	<i>D. marginatus</i>	Kazakhstan	100%	100%	8/60	43/56
Karaganda-3/98	RpA4	<i>D. marginatus</i>	Kazakhstan	100%	100%	9/37	38.7/30
Karaganda-5/98	RpA4	<i>D. marginatus</i>	Kazakhstan	90.8%	100%	7/45	15/54
Buryatiya-5/2000	DnS28	<i>D. silvarum</i>	Buryatiya	94.0%	82.0%	4.2/43.4	3.6/21.9
Shayman	DnS14	<i>D. silvarum</i>	Buryatiya	98.0%	100%	8/50	10/55
Doberman	RpA4	<i>D. reticulatus</i>	Omsk	90.0%	98.0%	3.4/29.6	7.7/35.9

tick species, this tick will both serve as a vector and a reservoir of the bacteria and the distribution of the rickettsiosis will be identical to that of its tick host. In *Dermacentor* ticks, *R. slovaca* multiplies in almost all organs and fluids of its tick host, particularly in the salivary glands and ovaries, which enables transmission of rickettsiae during feeding and transovarially, respectively.

Herein, all studied *R. raoultii* strains were maintained in laboratory cohorts of *Dermacentor* ticks for four to seven generations. All strains demonstrated a high level of transovarial and transstadial transmission. Rickettsial concentration in preimaginal stages before feeding was low, but we observed a considerable increase in rickettsial concentration following blood feeding. On the basis of our results, we demonstrated that all four studied *Dermacentor* tick species, *D. nuttalli*, *D. silvarum*, *D. marginatus* and *D. reticulatus*, are competent vectors of *R. raoultii*. Therefore, in Europe, *Dermacentor* ticks may be the efficient

vectors of *R. raoultii* and *R. slovaca*, both of which cause tick-borne lymphadenitis in humans.

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