衛生動物 (1995.09) 46巻3号:241～247.

# シュルツェマダニとライム病ボレリア感染マダニと非感染マダニにおける発育の比較

中尾 稔

[Jpn. J. Sanit. Zool. Vol. 46 No. 3 p. 241-247 1995]

# *Ixodes persulcatus* and Lyme disease spirochete: Comparison of development between infected and noninfected ticks

Minoru NAKAO*

*Department of Parasitology, Asahikawa Medical College, Asahikawa 078, Japan*

(Received: 12 April 1995; Accepted: 25 May 1995)

Key words: Lyme disease spirochete, *Ixodes persulcatus,* tick development.

**Abstract:** The ixodid tick, *Ixodes persulcatus,* is a principal vector for the Lyme disease spirochete, *Borrelia burgdorferi* sensu lato. To examine whether the infection affects the tick development, the amount of oviposited eggs, hatchability, molting rate from larvae to nymphs, and body sizes of nymphs and adults were compared between the infected and noninfected groups. No remarkable differences were observed in these parameters. Comparison was also made on the blood-feeding activity. The infected nymphs showed the same activity as the noninfected ones. These results suggest that the spirochete may act as a commensal in the tick host.

## INTRODUCTION

Ticks transmit viral, rickettsial, bacterial, and protozoal diseases to vertebrate hosts and the pathogenic microorganisms are related with ticks symbiotically. The pathogens must adapt to the various physiological changes of ticks during blood-feeding, blood-meal digestion, molting, and diapause. The infection, which can persist for the life span of ticks without any detrimental effects on ticks, is regarded as the most successful adaptation. Recently, ticks of *Ixodes ricinus, Ixodes scapularis, Ixodes pacificus,* and *Ixodes persulcatus,* which are grouped under the *I. ricinus* species complex, appeared to be vectors for the Lyme disease spirochete, *Borrelia burgdorferi* sensu lato, to humans. Most studies on interaction between the spirochete and its vectors have focused on transmission manner during feeding on vertebrate hosts (Benach *et al.,* 1987; Piesman *et al.,* 1987, 1991; Zung *et al.,* 1989; Nakayama and Spielman, 1989; Gern *et al.,* 1990; Piesman, 1993; Shih and Spielman, 1993). However, the influence of *Borrelia* infection on the development of ticks has not been investigated sufficiently.

In Japan, *I. persulcatus* serves as a tick vector for several *Borrelia* genospecies associated with Lyme disease (Nakao *et al.,* 1994). While conducting the field surveys, I noticed that deformation or undersized development of the unfed adult ticks were rare regardless of their high prevalence of spirochetal infection. However, nothing is known about the symbiotic relations between *Borrelia* species and *I. persulcatus.* In this report, comparative studies between the spirochete-infected and noninfected ticks were made by using field-collected and laboratory-reared *I. persulcatus.* The parameters examined were the amount of oviposited eggs, hatchability, molting rate, and body size. The blood-feeding activity was also investigated.

* 中尾　稔：旭川医科大学寄生虫学教室（〒078 旭川市西神楽4線5号3-11）

## Materials and Methods

*Hosts.* The Japanese white rabbit, *Oryctolagus cuniculus,* served as a host for adult *I. persulcatus.* The rabbits were purchased from Ichikawa Laboratory Co., Ltd. (Tokyo, Japan). The Mongolian jird, *Meriones unguiculatus,* reared in my laboratory was used as a host for larval and nymphal *I. persulcatus.* The rodent also served as a host for spirochetes.

*Spirochete isolate. Borrelia* isolate JEM6, which was derived from a patient of Lyme disease in Hokkaido (Fukunaga *et al.,* 1993), was used for infecting immature *I. persulcatus.* Previous study demonstrated that the tick and jird are highly susceptible to the isolate (Nakao and Miyamoto, 1994). The isolate has been maintained by alternate passage between ticks and jirds. The spirochete-infected nymphs were reared from larvae that had fed on jirds that had previously been bitten by infected nymphs.

*Culture.* To distinguish between infected and noninfected ticks, the triturated midgut of each tick was inoculated into 6 ml of BSK II medium (Barbour, 1984) containing rifampin (50 μg/ml) in a culture tube as described previously (Miyamoto *et al.,* 1992). The tubes were incubated at 31°C and examined for spirochetes by dark-field microscopy weekly for 4 weeks. In the case of replete females after laying eggs, the midgut and ovarian tissues were co-cultured.

*Field-collected adult ticks.* During May–June seasons in 1992 and 1993, unfed adult ticks of *I. persulcatus* were collected by flagging vegetation in Sibecha (43°21′N, 144°37′E) and Nemuro (43°15′N, 145°24′E), Hokkaido. The ticks were placed in large vials together with wet filter papers and kept at 4°C for 2–4 weeks until use.

*Laboratory-reared larval ticks.* The first filial generation larvae ($F_1$ progeny) of *I. persulcatus* were used for experiment. The larval colony originated from a wild-caught female in Sibecha that had fed to repletion on a rabbit in the laboratory. Prior to use, approximately 50 larvae were surface-sterilized, homogenized, and put into 6 ml of BSK II medium. Negative result of the culture indicate that the larval colony is free of inherited spirochetal infection.

*Study on fecundity.* The field-collected adult ticks in Sibecha were fed to repletion on a rabbit. Both of the rabbit ears were clothed with bags and 35 females were placed into each bag along with equal number of males. Six or seven days later, replete females were collected and weighed by using an analytical balance (A120S; Sartorius, Germany). Those were housed individually in plastic containers (60 by 30 by 15 mm), the bottoms of which were covered with hardened gypsum, and kept at 25°C in a saturated humidity with a photoperiod of 12:12 (L:D). Approximately 3 weeks after onset of oviposition, egg masses were weighed and each maternal tick was dissected for spirochetal culture. Fifty eggs randomly selected from each mass were transferred to another container and larval emergence was monitored weekly for 4 weeks.

*Study on molting.* A jird was infected with the isolate JEM6 by bites of 12 infected nymphs. Three weeks after nymphal feeding, approximately 300 laboratory-reared larvae were placed on the infected jird which was confined in a tube made of a wire net. Similarly, the larvae were allowed to feed on a normal jird as a negative control. Each jird was caged over a pan of water for 4 days. Of replete larvae dropped in water, 200 larvae were reared to the nymphal stage under the same condition as described above. After 8 weeks, molted nymphs were counted and 20 nymphal samples were dissected for spirochetal culture. The infected and noninfected nymphs obtained in this study were also used for the other studies on body size and blood-feeding activity described below.

*Study on body size.* The field-collected unfed adult ticks (100 males and 100 females) in Nemuro were dissected for spirochetal culture and the remaining tick bodies after dissection were individually preserved in 70% ethanol. Based on the result of culture, 20 infected males, 20 noninfected males, 20 infected females, and 20 noninfected fe-

males were selected at random. In addition, the laboratory-reared nymphs (19 infected and 20 noninfected) were used. These tick specimens were immersed in 10% KOH for 3 hr, rinsed with water, dehydrated with absolute ethanol, cleared with xylene, and mounted on glass slides with Canada balsam. The sites of tick body shown in Table 3 were measured by a microscopy with calibrated micrometer.

*Study on blood-feeding activity.* The JEM6-infected or noninfected nymphs were fed to repletion on jirds 8 weeks after molting. Numbers of jirds used were 4 for infected nymphs and 4 for noninfected nymphs. Twelve nymphs were placed on each jird as described above and the parasitized jirds were individually caged over pans of water. Nymphal repletion was observed for 5 days and dropped nymphs were counted.

*Statistical analysis.* The values obtained from studies on fecundity, body size, and blood-feeding activity were analyzed by Student's *t*-test. Chi-square test was used for the proportional data on molting. Simple linear regression was applied to determine if the body weight of replete females was correlated with the weight of oviposited eggs. All tests were conducted at $p<0.01$ level of significance.

## Results

### *Fecundities of infected and noninfected ticks*

Fifty-two replete females were recovered from a rabbit; however, 6 were died during rearing process. All survived ticks laid eggs. Of those, 7 were infected with spirochetes and 39 were noninfected. The mean body weight (±S.D.) of infected group was 508± 139 mg and that of noninfected group was 516±117 mg. Both values did not differ significantly. The mean egg weights of both groups were also identical. The infected and noninfected groups laid 278±94 and 293± 71 mg eggs, respectively. These results showed that the bloodmeal acquisition and oviposition were unaffected by spirochetal infection. Accordingly, the data of both groups were combined and the egg weight was regressed against the body weight. As shown in Fig. 1, the relationship between egg ($Y$) and body ($X$) weights was linear ($r=0.98$) and the formula for this regression was $Y=-34.44+0.63X$.

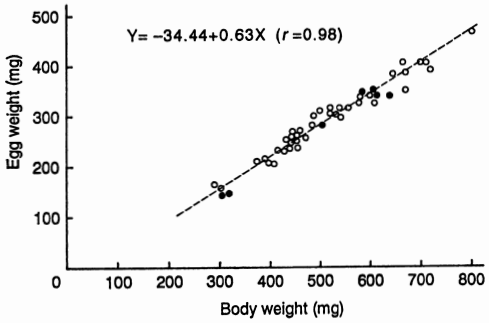


Fig. 1 Relationship between egg weight and body weight of replete females.
Closed circles, spirochete-infected females ($n=7$); open circles, noninfected females ($n=39$).

Table 1 Hatchability of eggs from spirochete-infected and noninfected maternal ticks of *I. persulcatus*.

| Spirochetal infection in maternal ticks | No. maternal ticks examined | % of hatched eggs* Mean±S.D. (range) |
|---|---|---|
| Infected | 7 | 98.0±2.3 (94–100) |
| Noninfected | 39 | 98.2±5.1 (70–100) |

* Fifty eggs from each maternal tick were examined.

The hatchability was compared between the infected and noninfected groups (Table 1). Larval emergence was observed in all batches of eggs and the rates of hatched eggs ranged from 70 to 100%. The rates of both groups did not differ significantly.

### *Molting rates of infected and noninfected ticks*

As shown in Table 2, 80.5% of replete larvae which had fed on the JEM6-infected jird molted to nymphs. Molting rate of noninfected larvae was 85.0%. Both values did not differ significantly. When examined for the molting rates (8 weeks after larval feed-

Table 2 Molting rates from larvae to nymphs in spirochete-infected and noninfected *I. persulcatus*.

| Blood sources for larvae | No. replete larvae reared | No. nymphs molted (%)* | Spirochetal culture | |
|---|---|---|---|---|
| | | | No. nymphs examined | No. positive (%) |
| Infected jird | 200 | 161 (80.5) | 20 | 19 (95.0) |
| Noninfected jird | 200 | 170 (85.0) | 20 | 0 ( 0 ) |

* Molting rates were observed 8 weeks after larval feeding.

Table 3 Comparison of body sizes between spirochete-infected and noninfected *I. persulcatus*.

| Measurement sites* | Mean±S.D. (μm) | | | | | |
|---|---|---|---|---|---|---|
| | Laboratory-reared nymphs | | Field-collected females | | Field-collected males | |
| | Infected ($n$=19) | Noninfected ($n$=20) | Infected ($n$=20) | Noninfected ($n$=20) | Infected ($n$=20) | Noninfected ($n$=20) |
| A | 466±21 | 461±21 | 1,105±58 | 1,109± 61 | 580±20 | 596±27 |
| B | 255± 6 | 247± 9 | 577±20 | 574± 33 | 367±14 | 363±16 |
| C | 887±39 | 872±38 | 1,811±92 | 1,847±114 | 1,596±95 | 1,604±62 |
| D | 143±12 | 144±10 | 354±25 | 362± 39 | 365±25 | 362±29 |
| E | 349±11 | 348±14 | 756±42 | 759± 33 | 607±24 | 611±26 |
| F | 703±23 | 688±29 | 1,468±71 | 1,468± 76 | n.m.** | n.m. |
| G | 619±22 | 602±17 | 1,250±77 | 1,243± 64 | n.m. | n.m. |

* A, length from apex of hypostome to posterior margin of basis capituli; B, width of basis capituli; C, length from posterior margin of basis capituli to anterior margin of anus; D, greatest diameter of spiracular plate; E, length of tarsus I; F, length of scutum; G, width of scutum. ** Not measured.

ing), all molted nymphs were alive and the remaining larvae seemed to be diapausing. The spirochete transmitted transstadialy from larvae to nymphs and the prevalence of infection in molted nymphs was 95.0%.

*Body sizes of infected and noninfected ticks*

The body sizes of field-collected unfed adults and laboratory-reared unfed nymphs were compared between spirochete-infected and noninfected groups (Table 3). Although various sites were measured, no significant differences were observed between both groups. None of the specimens showed signs of deformation and undersized development.

*Blood-feeding activities of infected and noninfected ticks*

Data on the blood-feeding activity of the JEM6-infected and noninfected nymphs were summarized in Table 4. Nymphal repletion terminated 3–4 days after exposure on jirds and no delays of feeding were observed in the infected nymphs. The mean numbers of replete nymphs recovered were identical between the infected and noninfected groups.

## Discussion

In *I. persulcatus* females, neither blood-meal acquisition nor oviposition were affected by spirochetal infection. Regardless of the maternal infection, the weights of oviposited eggs were highly correlated with the weights of engorged bodies immediately after detachment. This fact clearly indicates that the amount of nutriments acquired is an important factor for oocyte development. As concerns hatchability, no difference was observed between infected and noninfected groups.

Table 4 Comparison of blood-feeding activity between spirochete-infected and noninfected *I. persulcatus* nymphs.

| Nymphs | No. hosts used* | No. nymphs allowed to feed | No. replete nymphs recovered** Mean±S.D. (range) |
|---|---|---|---|
| Infected | 4 | 12 per host | 10.8±1.0 (10–12) |
| Noninfected | 4 | 12 per host | 10.5±1.3 ( 9–12) |

* The Mongolian jird was used as a host. ** Nymphal repletion terminated 3–4 days after exposure on hosts.

Schoeler and Lane (1993) obtained similar results in females of North American *I. pacificus* and concluded that the midgut-restricted spirochetal infection of maternal ticks did not affect the quantity or viability of eggs. The spriochetal infection in nymphal and adult stages of North American *I. scapularis* (formerly called as *Ixodes dammini* (Oliver *et al.*, 1993)) is generally restricted in the lumen of midgut diverticula before feeding and the systemic infection occurs during repletion (Benach *et al.*, 1987; Zung *et al.*, 1989; Burgdorfer *et al.*, 1988). In the developing oocytes of the replete females, *B. burgdorferi* destroyed the microvillar processes responsible for the formation of egg cuticle, suggesting that oocytes fail to mature (Burgdorfer *et al.*, 1989). However, the suppression of oviposition in infected *I. scapularis* has not been evaluated quantitatively. There are no data on the systemic infection in *I. persulcatus*. If the prevalence of ovarian infection is very low, most of infected females will lay eggs normally. As reported previously (Nakao and Miyamoto, 1992), the transovarial transmission of spirochetes from females to larvae was not detected in *I. persulcatus*, suggesting that the ovarian infection seldom occurs.

The replete larvae of *I. persulcatus* which had fed on the spirochete-infected jird were successfully molted to nymphs and the spirochetes transmitted transstadialy from larvae to nymphs. The molting rate of infected group was slightly lower than that of non-infected group; however, both rates did not differ significantly. The data indicate that the molting is unaffected by spirochetal infection. If the spirochetes multiplied unrestrictedly within the replete larvae, the molting rate would be reduced. Piesman *et al.* (1990) reported that *B. burgdorferi* multiplied rapidly in larval *I. scapularis* after repletion on infected hosts and its number decreased dramatically during molting. For this reason, they speculated that the reformation process of chitin within molting ticks depletes the amount of N-acetylglucosamine available for spirochetal development. Further studies are required to determine the physiological and biochemical factors which control the growth of spirochetes during molting process.

The body sizes of nymphal and adult *I. persulcatus* were measured for a comparison between infected and noninfected groups. The data indicate that the body sizes are unaffected by spirochetal infection. However, in this study the degree of infection was not taken into consideration. Tick individuals display a wide variation in densities of spirochetes (Burkot *et al.*, 1994). The possibility that heavily infected ticks may become undersized should be examined.

In systemically infected *I. scapularis*, *B. burgdorferi* was found in the synganglion (Benach *et al.*, 1987; Zung *et al.*, 1989). The infection of central nervous system may cause the behavioral abnormality. Since the blood-feeding seemed to be the most important behavior, a comparison of this behavior was made between infected and noninfected *I. persulcatus* nymphs. The result showed that the spirochetal infection did not weaken the blood-feeding activity. However, the infection of synganglion was not examined in this

experiment.

In conclusion, the data presented in this report strongly suggest that *Borrelia* species associated with Lyme disease may act as a commensal in *I. persulcatus*. The non-*I. ricinus* complex tick, *Ixodes ovatus*, is unsusceptible to *Borrelia* species transmitted by *I. persulcatus* (Nakao and Miyamoto, 1994) and it serves as a specific vector for *Borrelia japonica* (Nakao *et al.*, 1992; Kawabata *et al.*, 1993). Further studies are needed to understand the specificity of symbiosis between ticks and spirochetes.

ACKNOWLEDGEMENT

I thank Kazuhiro Nakaya (Animal Laboratory for Medical Research, Asahikawa Medical College) for providing the Mongolian jird.

REFERENCES

Barbour, A. G. (1984): Isolation and cultivation of Lyme disease spirochetes. *Yale J. Biol. Med.*, **57**: 521–525.

Benach, J. L., J. L. Coleman, R. A. Skinner and E. M. Bosler (1987): Adult *Ixodes dammini* on rabbits: A hypothesis for the development and transmission of *Borrelia burgdorferi*. *J. Infect. Dis.*, **155**: 1300–1306.

Burgdorfer, W., S. F. Hayes and J. L. Benach (1988): Development of *Borrelia burgdorferi* in ixodid tick vectors. *Ann. N.Y. Acad. Sci.*, **539**: 172–179.

Burgdorfer, W., S. F. Hayes and D. Corwin (1989): Pathophysiology of the Lyme disease spirochete, *Borrelia burgdorferi*, in ixodid ticks. *Rev. Infect. Dis.*, **11**: S1442–S1450.

Burkot, T. R., J. Piesman and R. A. Wirtz (1994): Quantitation of the *Borrelia burgdorferi* outer surface protein A in *Ixodes scapularis*: Fluctuations during the tick life cycle, doubling times, and loss while feeding. *J. Infect. Dis.*, **170**: 883–889.

Fukunaga, M., M. Sohnaka, M. Nakao and K. Miyamoto (1993): Evaluation of genetic divergence of borrelial isolates from Lyme disease patients in Hokkaido, Japan, by rRNA gene probes. *J. Clin. Microbiol.*, **31**: 2044–2048.

Gern, L., Z. Zhu and A. Aeschlimann (1990): Development of *Borrelia burgdorferi* in *Ixodes ricinus* females during blood feeding. *Ann. Parasitol. Hum. Comp.*, **65**: 89–93.

Kawabata, H., T. Masuzawa and Y. Yanagihara (1993): Genomic analysis of *Borrelia japonica* sp. nov. isolated from *Ixodes ovatus* in Japan. *Microbiol. Immunol.*, **37**: 843–848.

Miyamoto, K., M. Nakao, K. Uchikawa and H. Fujita (1992): Prevalence of Lyme borreliosis spirochetes in ixodid ticks of Japan, with special reference to a new potential vector, *Ixodes ovatus* (Acari: Ixodidae). *J. Med. Entomol.*, **29**: 216–220.

Nakao, M. and K. Miyamoto (1992): Negative finding in detection of transovarial transmission of *Borrelia burgdorferi* in Japanese ioxidid ticks, *Ixodes persulcatus* and *Ixodes ovatus*. *Jpn. J. Sanit. Zool.*, **43**: 343–345.

Nakao, M. and K. Miyamoto (1994): Susceptibility of *Ixodes persulcatus* and *I. ovatus* (Acari: Ixodidae) to Lyme disease spirochetes isolated from humans in Japan. *J. Med. Entomol.*, **31**: 467–473.

Nakao, M., K. Miyamoto and M. Fukunaga (1994): Lyme disease spirochetes in Japan: Enzootic transmission cycles in birds, rodents, and *Ixodes persulcatus* ticks. *J. Infect. Dis.*, **170**: 878–882.

Nakao, M., K. Miyamoto, K. Uchikawa and H. Fujita (1992): Characterization of *Borrelia burgdorferi* isolated from *Ixodes persulcatus* and *Ixodes ovatus* ticks in Japan. *Am. J. Trop. Med. Hyg.*, **47**: 505–511.

Nakayama, Y. and A. Spielman (1989): Ingestion of Lyme disease spirochetes by ticks feeding on infected hosts. *J. Infect. Dis.*, **160**: 166–167.

Oliver, J. H., Jr., M. R. Owsley, H. J. Hutcheson, A. M. James, C. Chen, W. S. Irby, E. M. Dotson and D. K. McLain (1993): Conspecificity of the ticks *Ixodes scapularis* and *I. dammini* (Acari: Ixodidae). *J. Med. Entomol.*, **30**: 54–63.

Piesman, J. (1993): Dynamics of *Borrelia burgdorferi* transmission by nymphal *Ixodes dammini* ticks. *J. Infect. Dis.*, **167**: 1082–1085.

Piesman, J., T. N. Mather, R. J. Sinsky and A. Spielman (1987): Duration of tick attachment and *Borrelia burgdorferi* transmission. *J. Clin. Microbiol.*, **25**: 557–558.

Piesman, J., G. O. Maupin, E. G. Campos and C. M. Happ (1991): Duration of adult female *Ixodes dammini* attachment and transmission of *Borrelia burgdorferi*, with description of a needle aspiration isolation method. *J. Infect. Dis.*, **163**: 895–897.

Piesman, J., J. R. Oliver and R. J. Sinsky (1990): Growth kinetics of the Lyme disease spirochete (*Borrelia burgdorferi*) in vector ticks (*Ixodes dammini*). *Am. J. Trop. Med. Hyg.*, **42**: 352–357.

Schoeler, G. B. and R. S. Lane (1993): Efficiency of transovarial transmission of the Lyme disease spirochete, *Borrelia burgdorferi*, in the western blacklegged tick, *Ixodes pacificus* (Acari: Ixodidae). *J. Med. Entomol.*, **30**: 80–86.

Shih, C. M. and A. Spielman (1993): Accelerated transmission of Lyme disease spirochetes by partially fed vector ticks. *J. Clin. Microbiol.*, **31**: 2878–2881.

Zung, J. L., S. Lewengrub, M. A. Rudzinska, A. Spielman, S. R. Telford and J. Piesman (1989): Fine structural evidence for the penetration of the Lyme disease spirochete *Borrelia burgdorferi* through the gut and salivary tissues of *Ixodes dammini*. *Can. J. Zool.*, **67**: 1737–1748.

## 摘　要

### シュルツェマダニとライム病ボレリア感染マダニと非感染マダニにおける発育の比較

シュルツェマダニとライム病ボレリアの共生関係を調査するため，感染マダニと非感染マダニにおいて，飽血雌成虫の産卵量，産出された卵の孵化率，幼虫から若虫への脱皮率，若虫と成虫の体サイズを比較した．また，若虫の吸血行動についても比較した．その結果，どの項目についても感染群と非感染群で統計学的有意差を見いだすことはできなかった．シュルツェマダニとライム病ボレリアの関係は，ボレリアの片利共生であると考えられた．