

# Seasonal Occurrence of *Rhipicephalus sanguineus* in Okayama Prefecture, Japan and Effect of Temperature on Development of the Tick

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**ABSTRACT.** The seasonal occurrence of *Rhipicephalus sanguineus* on dogs was examined at a kennel in Okayama Prefecture, Japan. The number of ticks suddenly decreased after treatment with an acaricide in late August. Small numbers of adults and nymphs were detected in September and October, then ticks were not seen on the dogs early in November, when the mean temperature was below 15°C. Then 3 dogs were found to be infested by some adult ticks toward the end of March, when the mean temperature was above 11°C. The effects of temperature on the oviposition and the development of the tick were examined under laboratory conditions. The larval and nymphal post parasitic period, the pre-oviposition period and the oviposition period were prolonged when the temperature was decreased from 37 to 23°C. The oviposition period was extremely long at 14°C, but the tick could not develop below 14°C. No eggs hatched below 14°C. The ability to attach and engorge of adult ticks was examined under cold conditions. Unengorged adult ticks could attach to rabbits on the ear which were kept in an outdoor kennel in October, November and March, however they could not engorge completely in November. They could not attach on rabbits from December to February. The longevity of the tick was also examined under low temperatures. Unengorged adults could attach and engorge on rabbits after kept at 12°C with 50% relative humidity (RH) for 140 days or 12°C with 50% RH for 40 days followed by 4°C with 50% RH for 100 days. These findings suggest that *R. sanguineus* could be established in Okayama Prefecture under optimum condition. — **KEY WORDS:** canine, Okayama, *Rhipicephalus sanguineus*, seasonal occurrence, temperature.

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*Rhipicephalus sanguineus* is one of the three host ticks which prefer dogs as host at each stage of life cycle, and widely distributed in the world. But in Japan there have been few reports on the distribution of *R. sanguineus* except those in Okinawa Prefecture [14]. An incidence of *R. sanguineus* at a kennel in Okayama Prefecture in Japan was reported in our previous paper and it is suspected that ticks were introduced from U.S.A. with the imported dogs and the tick population has been established in the kennel, though there is not sufficient evidence [10].

We have continuously observed the seasonal occurrence of ticks at the kennel and also examined the effects of temperature on the oviposition, development and survival of the tick under laboratory or natural conditions. The objective of the present study is to obtain information on the establishment of *R. sanguineus* at a kennel in Okayama Prefecture.

## MATERIALS AND METHODS

**Kennel:** The kennel with 22 hunting dogs, 10 adult males and 12 adult females, had suffered from tick infestation every summer since 1987 when a dog was first imported from U.S.A.. The kennel is located in a yard of the owner's house in Okayama city, Okayama, Japan. Four of the dogs had been introduced from U.S.A. and none from Okinawa Prefecture. The tick was identified as *R. sanguineus* in August 1994 [10]. All ticks were removed from the dogs and the kennel except for two delivery ones was sprinkled with propoxur (Bolfo 1% dust, Bayer Japan Ltd.) late in August, 1994.

**Observation:** Each dog was examined for tick infestation three times a month and the ticks were counted at least once a month from August 1994 to April 1995. Ticks found on dogs were removed and preserved in 70% ethanol for identification.

**Ticks:** The laboratory strain of *R. sanguineus* was originated from an engorged female obtained from a dog [10]. Since July 1994 ticks have been maintained on rabbits in our laboratory by a modified method of Fujisaki *et al.* [7]. Eggs or dropped engorged ticks were placed in plastic dishes without light at 23°C with more than 90% relative humidity (RH). The bottom of the dishes was covered with a strip of wet gauze to maintain RH more than 90%. Ticks in parasitic stages were put on the ear of a domestic rabbit, enveloped with a tubular bag made of elasticated support bandage (Flexigrip, FRA production, Italy) (ear bag method) at room temperature.

**Effects of temperature on the oviposition and development of the tick:** The effect of temperature on the periods of pre-oviposition and oviposition of adults, egg incubation, and post-parasitic stage of larvae and nymphs, were examined in incubators maintained at 5 fixed temperatures of 4, 14, 23, 30 and 37°C. Three engorged females, 10 to 30 engorged larvae and nymphs were used for the experiments. Eggs layed by a female at 30°C were divided into 5 experimental groups with equal weight. Ticks were observed for oviposition and development for every day, and the average duration of each developmental stage was calculated.

**Effects of temperature on the infestation of adults:** Five males and five females of *R. sanguineus* each were put on

the ear of domestic rabbits by the ear bag method. The rabbits were kept in an outdoor kennel in Yamaguchi University (Yamaguchi city, Yamaguchi Prefecture, Japan), in the middle of October, and near the end of November, December, January, February and in the middle of March respectively. The ticks were examined for ability to attach and engorge until day 14 of each infestation.

*Effects of temperature on the longevity of adults:* Unfed 10 males and 10 females were kept at (1) 12°C with 50% RH for 140 days, or (2) 12°C with 50% RH for 40 days followed by 4°C with 50% RH for 100 days, before being put on the ear of a rabbit at room temperature. The survival rate and ability of attachment and engorgement of ticks were examined.

## RESULTS

*Seasonal occurrence of ticks on dogs:* The number of ticks detected and temperature from July 1994 to April 1995 are shown in Table 1. The number of ticks suddenly decreased after treatment with propoxur late in August. A small number of adults and nymphs were detected in

September and October, and after then no ticks were seen on the dogs early in November when the mean temperature was below 15°C. Three dogs were infested by some males and females toward the end of March, when the mean temperature was above 11°C.

*The effects of temperature on the development, infestation and longevity of tick:* The relationship between temperature and the oviposition period and development of ticks is shown in Figs. 1 and 2, respectively. The post parasitic period of larvae and nymphs, and pre-oviposition period and oviposition period of engorged females were prolonged inversely proportional to decreased temperatures from 37 to 23°C. But ticks did not develop below 14°C. The pre-oviposition and oviposition periods were extremely prolonged at 14°C and eggs layed at this temperature did not hatch even kept at 30°C and degenerated. Ticks laid no eggs at 4°C. The incubation period of normal eggs layed at 30°C was also prolonged when the temperature was decreased from 37 to 23°C, but eggs did not hatch below 14°C. The ability to attach and engorge of adult ticks was examined under the cold winter condition at Yamaguchi University. Unengorged adult ticks could attach to rabbits

Table 1. The mean temperature and seasonal occurrence of *Rhipicephalus sanguineus* on dogs at the kennel in Okayama Prefecture

Year	Month	Time	Temperature <sup>a)</sup> (°C)	No. of Ticks Detected				
				Total	Ma	Fe	Ny	La
1994	July	E	29.7	NS	—	—	—	—
		M	30.5	+	NC	NC	NC	NC
		N	29.3	+	NC	NC	NC	NC
	August	E	31.5	+	NC	NC	NC	NC
		M	29.0	411	135	256	17	3
		N	28.6	367	54	73	120	0
	September	E	27.8	16	5	7	4	0
		M	24.2	+	NC	NC	NC	NC
		N	22.4	19	5	9	5	0
	October	E	21.1	1	0	1	0	0
		M	20.5	+	NC	NC	NC	NC
		N	15.9	+	NC	NC	NC	NC
	November	E	14.7	—	0	0	0	0
		M	15.3	—	0	0	0	0
		N	10.2	NS	—	—	—	—
	December	E	10.1	—	0	0	0	0
		M	6.5	NS	—	—	—	—
		N	7.7	NS	—	—	—	—
1995	January	E	5.9	NS	—	—	—	—
		M	3.7	NS	—	—	—	—
		N	4.7	—	0	0	0	0
	February	E	4.4	NS	—	—	—	—
		M	6.2	NS	—	—	—	—
		N	5.3	—	0	0	0	0
	March	E	7.0	NS	—	—	—	—
		M	8.7	—	0	0	0	0
		N	11.2	+	NC	NC	NC	NC
	April	E	11.5	163	128	35	0	0
		M	13.4	+	NC	NC	NC	NC
		N	15.7	+	NC	NC	NC	NC

a) The mean temperature of early, middle and near the end of each month in Okayama city. Ma: male, Fe: female, Ny: nymph, La: larva, E: early in each month, M: middle of month, N: near the end of each month, NS: not surveyed, NC: not counted

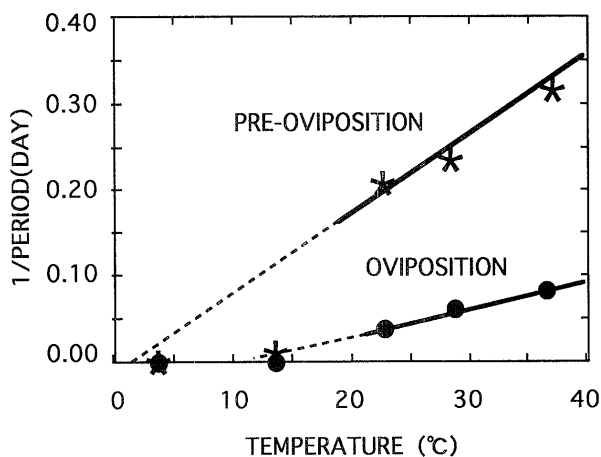


Fig. 1. Relationship between temperature and oviposition and pre-oviposition periods in engorged female of *Rhipicephalus sanguineus*. A circle and asterisk represent oviposition and pre-oviposition periods respectively.

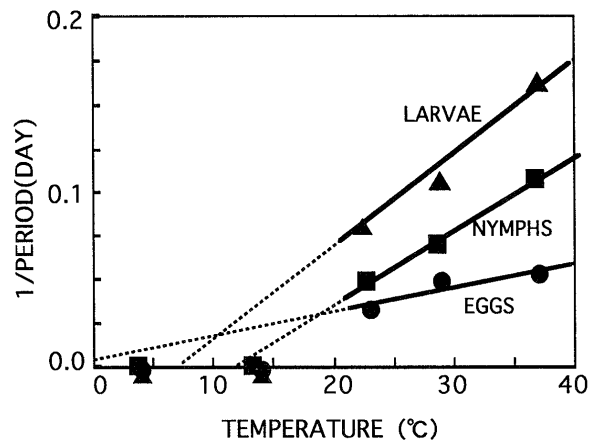


Fig. 2. Relationship between temperature and the periods of egg incubation and development of engorged larvae and nymphs of *Rhipicephalus sanguineus*. A circle, triangle, and square present incubation periods of egg and development periods of engorged larvae and nymphs, respectively.

on the ear which were kept in an outdoor kennel in October, November and March, however they could not engorge completely in November. The ticks could not attach on rabbits from December to February. The longevity of tick was also examined in low temperatures. All unengorged adults survived and could attach and engorge after kept at 12°C with 50% RH for 140 days. On the other hand, 8 males (80%) and 4 females (40%) survived and could attach and engorge after kept at 12°C with 50% RH for 40 days and subsequently at 4°C with 50% RH for 100 days.

## DISCUSSION

*Rhipicephalus sanguineus* has been hardly found in the prefectures of Japan and it seems to be introduced with pets and/or household effects of U.S. Forces personnel [14]. In our survey, 4 dogs in the kennel were imported from U.S.A. since 1987 and since then the kennel has suffered from tick infestation every summer. It is suspected that ticks were introduced from U.S.A. with the imported dogs and the tick population has been established in the kennel, though there is no sufficient evidence [10].

In the present study, we have continuously observed the occurrence of tick at the kennel from July 1994 to April 1995. All ticks were removed from the dogs and propoxur, an acaricide, was sprinkled on the kennel in late August, 1994. However ticks were still seen on September and October. Ticks might survive on two delivery kennels, which were not treated with the acaricide and occupied by puppies at that time. Ticks were not seen on the dogs early in November when the mean temperature was below 15°C, however, 3 dogs were found to be infested with some male and female ticks near the end of March, when the mean temperature was above 11°C. Mumcuoglu *et al.* [12] examined the seasonal occurrence of *R. sanguineus* in Israel and found a significant positive correlation between temperature and tick population size. As the species of tick is of tropical origin [14], temperature is thought to be the

most important factor for the occurrence of the tick in Okayama Prefecture. None of the dogs had been introduced from U.S.A. or Okinawa Prefecture during the monitoring period, so it is suspected from the results that adults of *R. sanguineus* had survived the winter season in the kennel during winter in Okayama Prefecture, because the tick population can be established in kennels even in the northern parts of U.S.A. [8].

To confirm this, we also examined the effects of temperature on the development and survival of the ticks under laboratory condition. Tick could well develop between 23 and 37°C but not below 14°C. The pre-oviposition and oviposition periods of engorged females were extremely prolonged at 14°C. Eggs laid at 14°C degenerated and did not hatch even kept at 30°C. Ticks laid no eggs at 4°C. The incubation period of normal eggs layed at 30°C was also prolonged when temperature was decreased from 37 to 23°C, but eggs did not hatch below 14°C. These findings suggest that *R. sanguineus* could not develop in Okayama Prefecture from November to April when the mean temperature was below 14°C. There are some informations about the laboratory conditions that affect the development and oviposition of *R. sanguineus* [7, 9, 11, 13]. Temperature below 20°C markedly made the pre-oviposition and oviposition periods prolong, and a constant temperature of 15°C was detrimental to egg production [13]. Larval ticks could molt between 18 and 38°C [9]. These informations also support the idea that *R. sanguineus* could not develop in Okayama Prefecture in winter. The sensitivity to temperature is quite different in different species of ticks. The ticks common in Japan, such as *Haemaphysalis longicornis*, *H. flava*, and *Ixodes ovatus* are more resistant to the cold conditions [3, 5, 6].

Unengorged adult ticks could attach to rabbits on the ear which were kept in an outdoor kennel in October, November and March, however they could not engorge completely in November. They could not attach to rabbits on the ear from December to February, so winter season seems too cold for

the ticks to attach to animals. The experiments were conducted in Yamaguchi University which is located in the same Chugoku district as of Okayama Prefecture. The longevity of the tick in low temperature was also examined. Some unengorged adults could attach to rabbits and engorge after kept at 12°C with 50% RH for 140 days and also in such more severe condition as 12°C with 50% RH for 40 days followed by 4°C with 50% RH for 100 days. This result suggested that adult *R. sanguineus* can survive the winter conditions in Okayama Prefecture. Survival periods are determined by both temperature and humidity [1]. The combination of optimal temperature and humidity may make the ability of ticks to attach and engorge keep longer even in Okayama Prefecture. These findings suggest that *R. sanguineus* can be established in Okayama Prefecture under optimal condition.

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