Seroepidemiological Study of *Coxiella burnetii* in *Cervus nippon* in Northern Japan

Morihiro YASUMOTO, Tsuyoshi YANASE,
Yasukazu MURAMATSU, Chiharu MORITA and Hiroshi UENO

1)Department of Veterinary Public Health, Rakuno Gakuen University,
581-2 Midori-machi Bunkyo-dai
Ebetsu, Hokkaido 069, Japan
(1997.2.21 received, 1997.5.6 accepted)

北日本のシカにおけるQ熱の抗体調査

安本 守宏, 柳 瀬 毅, 村松 康和, 上野 弘志, 森田 千春

1) 酪農学園大学獣医学部獣医公衆衛生学教室 〒069 北海道江別市文京台緑町582-1

ABSTRACT. Q-fever, caused by *Coxiella(C) burnetii*, is one of the most important zoonoses. In the present study, the antibody against *C. burnetii* was analyzed by indirect immunofluorescence antibody (IFA) test in 168 individuals of Japanese deer (*Cervus nippon*) to determine the prevalence of Q-fever in wild deer of northern Japan. Specific antibody against *C. burnetii* was found in 72(42.9%) deer, including 24 of 46 (52.2%) in Hokkaido and 48 of 122(39.3%) in Tohoku. In Toya and Shari, the positive rate between adult(66.7%) and immature animals(17.6%) was significant ($p<0.01$). In adult deer, a significant difference was also noted between Hokkaido and Tohoku($p<0.01$).

Key words : Zoonosis, Q fever, *C. burnetii*, Deer, FA

Jpn. J. Zoo Wildl. Med. 2(2): 101-106, 1997

INTRODUCTION

Q-fever, caused by *Coxiella(C) burnetii*, is one of the most important zoonoses in Japan. In most continents the main reservoirs of *C. burnetii* are ruminants, particularly cattle, sheep, goats and camels. *C. burnetii* is discharged from these animals mingled with amniotic fluid, placenta, milk and urine, and some kinds of ticks receive the microorganism by biting these animals. Because of its strong resistance to physical and chemical agents, the microorganism can be virulent a long time in the environment by slipping into dust.

In other animals, *C. burnetii* infection may be derived from dust containing the microorganisms or carried in tick bite, as well as directly from animal to animal. Sometimes *C. burnetii* infects carnivores such as dogs that eat the placenta and other uterine discharges of aborting animals [1]. The symptoms are fairly mild or inapparent, although abortion may result in sensitive animals [2]. In the human, the organism causes a serious influenza-like syndrome or atypical pneumonia, and in some persons who have a fundamental malady such as hepatitis or endocarditis, the disease progresses to chronicity [3].

It has been said that there is no Q-fever in Japan, but in

1952, 22 of 756 veterinarians (2.9%) in 3 of 18 prefectures were reported to have complement fixing (CF) antibody in the lower range of antibody titers, as found by research requested by WHO in the 1950s [4]. Since then, there has been no report of Q-fever in Japan. In recent years, however, it has become a common viewpoint that, for a while, Q-fever was spreading simultaneously with outbreaks of influenza in Japan.

Ejercito et al. [5] reported that Q-fever in wild animals has been spreading in Japan and a high antibody positive rate (68.9% in Hokkaido, 55.6% in Honshu) was recorded in Japanese deer (*Cervus nippon*) in 1993. Deer are not only one of the main wild ruminants in much of the northern hemisphere but they are also considered to be one of the main wild reservoirs of *C. burnetii*. In the present study, the antibody against *C. burnetii* was analyzed in *Cervus nippon* of northern Japan to determine the prevalence of Q-fever in wild deer in this region.

MATERIALS AND METHODS

Blood samples were collected at random from 168 wild and farmed healthy deer in the northern part of Japan from 1991 to 1996. Details regarding the geographical region and the age, sex and environment of the animals are tabulated in

Table 1. We collected 46 of the blood samples from immature and adult deer in Hokkaido prefecture (42° to 45°N, 140° to 145°E) from 1995 to 1996 in cooperation with animal ecologists. Of those 46 animals, 10 samples were collected from wild deer in Shari. One from straying wild deer in Ebetsu, and the other 25 were from deer isolated on a small island in Toya Lake and, therefore, may be considered semi-wild. In Hokkaido, sampling from these 36 deer, including immature and adult animals was done prudently under ketamine anesthesia so as to prevent the deer from stress when they were captured for a routine survey of the population. All wild deer were returned to the wild after physical examination and the setting of ear tags.

The rest 10 of the 46 samples from adult deer were collected from a private farm and a zoo in environs of Sapporo, in conjunction with a physical examination conducted on each animal. These deer were raised in a dense deer population. After centrifuge, these blood samples were stored at -4°C as serum.

In addition to the 46 samples from Hokkaido, 122 adult deer blood samples collected in the Tohoku region, (Miyagi, Iwate, and Aomori: 38° to 41°N, 140° to 142°E) from 1991 to 1993 were provided as sera by the Shichinohe Research Unit, National Institute of Animal Health, Tsu-

Table 1. Characteristics of the 168 deer examined

Region(n) ^{*1}	Number of samples			Environment
	Breakdown ^{*2}			
Hokkaido prefecture (46)				
Toya (25)	m 8	f 8	i 9	semi-wild
Shari (10)	m 2	f 0	i 8	wild
Ebetsu (1)	m 0	f 1	a 0	wild
Private farm (6)	m 1	f 5	a 0	farmed
Zoo (4)	m 1	f 1	a 2	farmed
Tohoku region (122)				
Sanriku (24)	m 24	f 0	a 0	wild
Kahoku (7)	m 7	f 0	a 0	half-farmed
Narugo (77)	m 9	f 10	a 58	half-farmed
Aomori (14)	m 12	f 2	a 0	half-farmed

*¹(n) : indicates number of samples from each region.

*²m : male, f : female, i : immature, a : adult but sex is not clear.

All samples except those of Toya and Shari are from adult deer serum.

kuba. The Tohoku batches included 24 from wild deer in Sanriku and 98 from farmed deer (Table 1). The farmed deer were raised in enclosed forestland in a low deer population (half-farmed).

Antibody against *C. burnetii* was detected by indirect immunofluorescence antibody (IFA) test on the sera of all the deer investigated in this experiment [6]. First, the sera of the 46 deer in Hokkaido were titrated as serial twofold dilutions from 8-to-1,024-fold, and the results were read at 1,000 $\times$ with a fluorescence microscope (OLYMPUS model BHF, Tokyo). The end-point titer recorded was the highest dilution with distinct fluorescing Rickettsiae. In this way, the distribution of antibody titers was determined for the deer sera. Accordingly, the 122 sera of Tohoku deer were screened by IFA, and the results were regarded as evidence of past infection. Nine Mile II BGM strain was used as antigen, and fluorescein-labeled affinity purified antibody to deer IgG (heavy chain+light chain, Kirkegaard & Perry Laboratories Inc. Maryland) was used as secondary antibody.

C. burnetii-positive rates were calculated from the sera of all animals sampled, including both wild and farmed deer. Significant differences of the antibody-positive rates between wild and farmed deer and between Hokkaido and Tohoku deer were determined by χ^2 test.

In Toya and Shari, where the sex and age of the captured deer were recorded, the antibody-positive rate was calculated in male and female deer. The statistical significance was determined by χ^2 test. In addition, positive rates were calculated in adult (over 1-year-old) deer. In the adult deer, significant differences of antibody-positive rates between Hokkaido and Tohoku deer and between wild and farmed animals were calculated in the same way.

RESULTS

Antibody titers of the Hokkaido deer sera tested against *C. burnetii* are shown in Table 2. The IFA titers formed a normal distribution pattern varying from 64 to greater than 1,024, with 256 occurring the most frequently against *C. burnetii*. Serum with a titer of 1:64 or greater was taken to be specific antibody-positive against *C. burnetii*.

Statistical interpretations of the positive rates are indicated in Table 4. Of the 168 deer sampled, 72 (42.9%) had specific antibody against *C. burnetii* Nine Mile II. Geographically, 24 of 46 (52.2%) samples in Hokkaido prefecture and 48 of 122 (39.3%) samples in Tohoku were antibody-positive. No significant difference was noted between the prevalence of *C. burnetii* in Hokkaido deer and Tohoku deer ($p>0.05$).

With regard to the animals' environment, 29 of 60 (48.3%) wild and semi-wild deer, and 43 of 108 (39.8%) farmed and half-farmed deer had specific antibody against *C. burnetii*. The difference was not statistically significant ($p>0.05$).

In Toya and Shari, 12 of 18 (66.7%) wild adult deer and 3 of 17 (17.6%) wild immature deer had specific antibody against *C. burnetii*. The difference between adult deer and immature deer in these regions was significant ($p<0.01$). Further comparison of adult deer disclosed that 21 of 29 (72.4%) adult deer in Hokkaido and 48 of 122 (39.3%) adult deer in Tohoku had specific antibody against *C. burnetii*. The difference was significant between these two groups ($p<0.01$).

Subdividing the adult animals still further, I found that 13 of 19 (68.4%) adult semi-wild and wild deer in Hokkaido and 8 of 10 (80.0%) farmed adult deer in Hokkaido had

Table 2. Antibody titers of deer sera tested against *C. burnetii*

	Titer of deer serum							
	>8	16	32	64* ¹	128	256* ²	512	1,024<
Number of sera	0	12	10	2	5	11	3	3

*¹Titer of 1:64 or greater was taken to be positive

*²1 : 256 occurring the most frequently against *C. burnetii*.

Morihiro YASUMOTO et al.

Table 3. Prevalence of antibody against *C. burnetii* in sera of deer in northern Japan

Region	Number of samples					Number of positive antibody responses to <i>C. burnetii</i> antigen						
	Total	Subtotal	Breakdown*				Total	Subtotal	Breakdown*			
Hokkaido prefecture												
	168	46					72	24				
Toya			m 8	f 8	i 9	t 25			m 6	f 6	i 2	t 14
Shari			m 2	f 0	i 8	t 10			m 0	f 0	i 1	t 1
Ebetsu			m 0	f 1	a 0	t 1			m 0	f 1	a 0	t 1
Private farm			m 1	f 5	a 0	t 6			m 1	f 3	a 0	t 4
Zoo			m 1	f 1	a 2	t 4			m 1	f 1	a 2	t 4
Tohoku region												
		122						48				
Sanriku			m 24	f 0	a 0	t 24			m 13	f 0	a 0	t 13
Kahoku			m 7	f 0	a 0	t 7			m 1	f 0	a 0	t 1
Narugo			m 9	f 10	a 58	t 77			m 2	f 6	a 21	t 29
Aomori			m 12	f 2	a 0	t 14			m 4	f 1	a 0	t 5

*m : male, f : female, i : immature, a : adult but sex is not clear, t : total

All samples except those of Toya and Shari are adult deer serum

Table 4. Q-fever antibody-positive rates in deer in northern Japan

Serum used	Positive rate		Comparative Positive rate		χ^2 test
All deer	Hokkaido prefecture	52.2%	Tohoku region	39.3%	p>0.05
	Wild deer	48.3%	Farmed deer	39.8%	p>0.05
Deer in Toya and Shari	Adult deer	66.7%	Immature deer	17.6%	p<0.01*
All adult deer	Hokkaido prefecture	72.4%	Tohoku region	39.3%	p<0.01*
Adult deer in Hokkaido	Wild deer	68.4%	Farmed deer	80.0%	p>0.05

*Significant : p<0.05

specific antibody against *C. burnetii*. The difference was insignificant between wild and farmed adult deer (p>0.05).

DISCUSSION

In this study, *C. burnetii* infection in northern Japan was found to be more prevalent in deer than in cattle about (15.0% to 30.0%) or in human (about 3.0%), an important result that is in agreement with reports in the literature 5,7. Furthermore, in contrast to reports to date, the geographical location of the infected deer was clarified, evidencing *C. burnetii* infection is widespread in northern Japan.

As with many infectious diseases, specific antibody against *C. burnetii* in immature deer was significantly lower than that in adult deer. It is considered that the lesser

accumulation of opportunistic infection protects immature deer from pathogenic microorganisms, and consequently specific antibody rates become lower.

The antibody-positive rate in the Hokkaido deer, including many immature animals, was not significantly higher than that of Tohoku deer comprising adult animals only. Accordingly, the rate of sera testing specific antibody-positive against *C. burnetii* in adult deer was significantly higher in Hokkaido than in Tohoku. The reason for increased *C. burnetii* among deer in northern Japan may be attributable to the strong resistance of *C. burnetii* to physical and chemical agents, the endurance of microorganisms, and the weakened mucosa of animals in freezing weather, a condition that seems to increase the animal's susceptibility to infection. This suggests the possibility of seasonal changes

Coxiella burnetii in *Cervus nippon*

in Q-fever. Further study is necessary.

In Hokkaido, specific antibody-positive rate against *C. burnetii* of farmed adult deer (80.0%) was slightly higher than that of wild adult deer (68.4%) but the difference was insignificant. The rate of positivity may be associated with the deer population density, as the opportunity for infection in the farmed deer population is usually greater than that of wild deer. In this study, however, most sera of wild deer in Hokkaido were sampled from isolated animals in small island, so this trend was not confirmed clearly. Further study is necessary because in Japan the farming of wild ruminants is still in its infancy. The difference in positivity between wild and farmed deer might be an index of the prevalence of Q-fever in the forest ecosystem because there are few reports of Q-fever in large wild ruminants in Japan although there are some reports of the disease in stock animals and companion animals [4, 7, 8].

The results of this study suggest that human activity gives rise to the prevalence of Q-fever. Along this line, the Yezo Wolf (*Canis lupusrex*, native species of Hokkaido) was exterminated by humans by 1942, and this greatly influenced the increase in the deer population [9]. By general consensus of animal ecologists, there used to be many more deer in Hokkaido before cultivation by humans. Both an increased deer widespread and cultivation contributed not only to hunger in deer but also to the opportunity for contact with cows and humans. As the deer that were bereaved of their habitat come to farmland or pasture every day and night to get food, agricultural losses caused by deer in Hokkaido have been increasing year by year. Such loss is estimated to be more than four billion yen ("Inhabit, capture and damage conditions etc. of *Cervus nippon yesoensis*," private communication used by permission from the Hokkaido Government, Department of Health and Environment, Office of Environmental Affairs, Nature Preservation Division).

Seven cases of Q-fever infestation from deer to dog to man were reported in 1989 in New Brunswick [1]. This shows the need for human awareness that people are confronted with the opportunity to contract *C. burnetii* from deer.

Theoretically, excessive human activities in nature bring upon man his own decline. Not only from the common viewpoint that Q-fever is considered an import disease, but also as suggested by the present results, human behavior is thought to play an undeniable role in the spread of Q-fever. The study bears out the correlation that, in northern Japan, *C. burnetii* infection in deer is increasing with the acceleration of human activities.

要 約

Q熱は重要な人獣共通感染症の中の一つで、リケッチアの1種 *Coxiella(C) burnetii* がその病原体である。本研究では、北日本に生息する168頭のシカを対象に、間接蛍光抗体法を用いて *C. burnetii* Nine Mile II 相菌に対する特異的抗体の検出を行った。北海道の46頭のシカのうち24頭の52.2%、東北地方の122頭中48頭の39.3%、合わせて168頭中72頭の42.9%から特異的抗体が検出された。また、北海道内の斜里町および洞爺村のシカ46頭のうち、成獣では18頭中12頭の66.7%、1歳未満の幼獣では17頭中3頭の17.6%が抗体陽性で、両者間には有意差が認められた。そこで、成獣のみを対象として比較したところ、*C. burnetii* 抗体陽性率は北海道の29頭中21頭(72.4%)に、東北地方の122頭中48頭(39.3%)に検出され、北方で高くなる傾向がみられた。

キーワード：人獣共通感染症, Q熱, *Coxiella burnetii*, ニホンジカ, 間接蛍光抗体法

ACKNOWLEDGEMENTS

I wish to thank Dr. K. Kaji, Nature Conservation Department, Hokkaido Institute of Environmental Sciences, for accompanying me on the sampling excursions, and Mr. M. Yamanaka and H. Okada, Shiretoko Nature Center, Shari City, for giving me the privilege of sampling blood from the deer, and, Mr. T. Imada and Mr. H. Murata, Shichinohe Research Unit, National Institute of Animal Health, Dr. E. Isogai, Department of Preventative Dentistry, Health Science University of Hokkaido, Dr. T. Mukai, Sapporo Maruyama Zoo, Y. Takahashi, Department of Theriogenology, Faculty of Veteri-

Morihiro YASUMOTO et al.

na-y Medicine, Hokkaido University, for providing me with deer serum samples. I also express my sincere thanks to Prof. N. Kennedy for reading the manuscript.

REFERENCES

1. Thomas L, David W, Jim W, Thomas M. 1991. Q-fever: from deer to dog to man. *Lancet* 337: 676-677.
2. Sanford SE, Josephson GKA, Macdonald A. 1994. *Coxiella burnetii* (Q-fever) abortion storms in goat herds after attendance at annual fair. *Can Vet J* 35: 376-378.
3. Palmer SR, Young SEJ. 1982. Q-fever endocarditis in England and Wales. *Lancet* 1982(2): 1448-1449.
4. Kitaoka M. 1954. Q-fever. *J Publ Health*. 15: 67-73 (in Japanese).
5. Ejecito CL, Cai L, Htwe KK, Taki M, Inoshima Y, Kondo T, Kano C, Abe S, Shiota K, Sugimoto T, et al. 1993. Seroepidemiology of *Coxiella burnetii* infection in wild animals in Japan. *J Wildl Dis* 29(3): 481-484.
6. Morita C, Tsuboi Y, Iida A, Mohri S, Handa S, Fuyuki M. 1989. Spotted fever group rickettsia in dog in Japan. *Jpn J Med Sci Biol* 42: 143-147.
7. Yoshiie K, Oda H, Nagano N, Matayoshi S. 1991. Serological evidence that the Q-fever agent (*Coxiella burnetii*) has spread widely among dairy cattle of Japan. *Microbiol Immunol* 35: 577-581.
8. Htwe KK, Amano K, Sugiyama Y, et al. 1992. Seroepidemiology of *Coxiella burnetii* in domestic and companion animals in Japan. *Vet Res* 131: 490.
9. Higashi S, Kondo N, et al. 1993. Biodiversity and ecology in the northernmost Japan. In *Mammal Fauna and Its Distribution in Hokkaido* (Hokkaido University Press): 76- 87.