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Positive rates of anti-acari-borne disease antibodies of rural inhabitants in Japan

Tsutomu TAKEDA[1)]*, Hiromi FUJITA[2)], Masumi IWASAKI[3)], Moe KASAI[3)], Nanako TATORI[4)], Tomohiko ENDO[5)], Yuuji KODERA[1,5)] and Naoto YAMABATA[6)]

[1)]Wildlife Section, Center for Weed and Wildlife Management, Utsunomiya University, 350 Mine, Utsunomiya-shi, Tochigi 321-8505, Japan
[2)]Mahara Institute of Medical Acarology, 56-3 Aratano, Anan-shi, Tokushima 779-1510, Japan
[3)]Nikko Yumoto Visitor Center, National Parks Foundation Nikko Branch, Yumoto, Nikko-shi, Tochigi 321-1662, Japan
[4)]Department of Agriculture, Utsunomiya University, 350 Mine, Utsunomiya-shi, Tochigi 321-8505, Japan
[5)]United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan
[6)]Institute of Natural and Environmental Sciences, University of Hyogo, Sawano 940, Aogaki-cho, Tanba, Hyogo 669-3842, Japan

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ABSTRACT. An assessment of acari (tick and mite) borne diseases was required to support development of risk management strategies in rural areas. To achieve this objective, blood samples were mainly collected from rural residents participating in hunting events. Out of 1,152 blood samples, 93 were positive against acari-borne pathogens from 12 prefectures in Japan. Urban areas had a lower rate of positive antibodies, whereas mountainous farming areas had a higher positive antibody prevalence. Residents of mountain areas were bitten by ticks or mites significantly more often than urban residents. Resident of mountain areas, including hunters, may necessary to be educated for prevention of akari-borne infectious diseases.

KEY WORDS: acari-borne disease, mountainous farming area, wildlife management

Effects of wild boars, deer, bears and monkeys on agriculture and forestry have steadily increased in Japan. Therefore, the Government of Japan started a program to halve the number of deer and wild boars by 2023 [8]. Then, it was necessary to capture the mammals to achieve the population management goals. However, it was difficult to achieve the target because of an aging and decreasing hunter population [17, 18]. In response, local governments across Japan increased the frequency of hunting license examination preliminary workshops to develop new hunters. It is acknowledged that hunters must take various risks into consideration when interacting with wild animals. Use of guns and traps requires attention because these tools could kill the victim. However, there are other risks less visible; when hunters enter forests or thickets, they risk being bitten by ticks and exposing themselves to acari-borne diseases.

The acari-borne diseases endemic among hunters, such as tularemia, have a long history of zoonosis [9]. After World War II, it was reported that a hunter catching a rabbit and the hunter's family member who cooked and ate the rabbit were infected with tularemia [11, 12]. Recently, the number of hunters targeting the Japanese hare *Lepus brachyurus* has decreased and the number of wild hares has also decreased [1, 15]. Due to reduced interaction with hares, the number of cases of the infectious disease decreased among hunters. As the incidence of tularemia decreased, there was less concern for acari-borne diseases among hunters who work in the mountains until an incidence of Severe Fever with Thrombocytopenia Syndrome (SFTS). However, there were still several acari-borne diseases endemic to Japan such as Japanese spotted fever and tularemia [6, 13].

To effectively develop risk management strategies for tick-borne diseases in rural areas we need to have a good assessment of the current status of diseases and their prevalence. In this research, blood samples were collected mainly from hunters and local government officers that conducted wild animal management from 2016 to 2018. We evaluated the tick-borne disease risk using presence of positive antibodies and classified the samples based on an assessment of tick or mite bite experiences using a questionnaire. This project received approval that it "met ethical considerations for research using humans" from Utsunomiya University (registration number H15-0051).

The investigation areas included the hilly rural areas in the following prefectures: Iwate, Fukushima, Tochigi, Yamanashi, Mie, Wakayama, Gifu, Hyogo, Shimane, Saga, and Okinawa. In accordance with the right of each test subject to maintain their rights in

*Correspondence to: Takeda, T.: ixodes@cc.utsunomiya-u.ac.jp
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Table 1. The number of positive samples for each infectious agent: Japanese spotted fever (*R. japonica*), murine typhus (*R. typhi*), Tsutsugamushi disease (*O. tsutsugamushi* serotypes), SFTS virus and tularemia (*F. tularensis*)

Prefectures	Tested number	Positive number	Rate (%)	*Rickettsia japonica*	*Rickettsia typhi*	*Orientia tsutsugamushi*						SFTS	*Francisella tularensis*
						Gilliam	Karp	Kato	Irie/ Kawasaki	Hirano/ Kuroki	Shimokoshi		
Iwate	60	5	8	2	nd	nd	3	nd	3	4	4	nd	1
Fukushima	94	1	1	1	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tochigi	217	27	12	13	1	1	4	2	1	4	7	nd	4
Yamanashi	13	1	8	nd	nd	nd	nd	nd	nd	nd	nd	nd	1
Gifu	24	2	8	nd	nd	nd	nd	nd	nd	1	1	nd	nd
Mie	98	6	6	3	1	nd	nd	nd	nd	nd	1	nd	2
Wakayama	128	5	4	2	2	nd	nd	nd	nd	nd	nd	1	nd
Hyogo	61	1	2	1	nd	nd	nd	nd	nd	nd	nd	nd	nd
Shimane	258	25	10	12	6	5	3	1	nd	4	4	1	1
Saga	92	9	10	1	5	4	1	nd	3	2	2	nd	nd
Nagasaki	57	9	16	6	2	nd	nd	1	nd	nd	nd	nd	2
Okinawa	26	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Others (10)	24	2	8	nd	nd	nd	1	nd	nd	1	nd	nd	1
Total	1,152	93	8	41	17	10	12	4	7	16	19	2	12

nd: no detected. Others (10): blood samples from other prefectures: 4 samples from Saitama and Fukuoka, 2 from Hokkaido, Ibaraki, Gunma, Tokyo, Kanagawa, Aichi, Hiroshima and Kumamoto.

medical studies, we obtained informed consent from each participant for this research. Once the candidates understood the purpose of the research, they signed the consent form to participate in the blood tests. The consent form requested the participant to write their name, address, age and gender.

Their address data were classified according to the list of agriculture areas 2017, prepared by the Ministry of Agriculture, Forestry and Fisheries of Japan. All the participants' residences were divided into the following four categories: Mountainous farm area, Hilly farm area, Flat farm area and Urban area. They were classified as former cities, wards, towns, and villages based on fundamental criteria (e.g. the rate of cultivated land or forest land, grazing land area, or gradient of farmland) that define the structure of agriculture areas [7]. The questionnaire on tick awareness was implemented in Tochigi, Gifu, Mie, Wakayama, Hyogo and Wakayama since February 2018. The question was: How many times have you been bit? (no bites: 0 times, 1–4 times or more than five times). This survey was a signed investigation.

Blood was sampled from 1,152 people from Iwate, Fukushima, Tochigi, Mie, Wakayama, Hyogo, Shimane, Saga, Nagasaki and Okinawa between 2015 to 2018. Blood was collected in 3 ways: 1) From 2015 to 2016 physicians and nurses collected 5 m*l* blood from the upper arm using EDTA vacuum vials from 99 people from Tochigi, Saga, and Okinawa; 2) From January to March 2017, the filter-paper technique, which was adopted for blood collection [10], was used for 153 people from Tochigi and Fukushima. A portable blood-collection filter-paper unit (Asahi Polyslider, Inc., Osaka, Japan) was used so that many people could collect blood at once and there were few burdens to the test subject. The filter paper with the collected blood was cut into 8 mm diameter circles so that the amount of blood analyzed was set to 20 μl; it was immersed in phosphate buffer (pH 7.4) and the serum was extracted; 3) Since 2017 the "Kantan Saiketsu Set (easy-to-use kit)" (Eiken Chemical Co., Inc., Tokyo, Japan) was adopted, which is a self-collection technique designed by Kamaura *et al*. [2]. Approximately 30 μl of plasma was collected after centrifuge and stored at −20°C until the antibody-titer test was conducted. This method was used to collect blood from 901 people from Iwate, Tochigi, Mie, Wakayama, Hyogo, Shimane, Saga, Nagasaki and 11 other prefectures (Table 1).

The antibody titers for several pathogens were scaled according to the following methods. The rickettsia antigen used each of the following rickettsia stocks proliferated in L929 cells: Japanese spotted fever (*Rickettsia japonica*, Aoki stock), murine typhus (*Rickettsia typhi*, Wilmington stock), and Tsutsugamushi disease (*Orientia tsutsugamushi*: Gilliam sub-type was Kaisei strain of Japanese Gilliam, Karp sub-type was Japanese Karp-2 type Sato strain, and Kato, Irie/Kawasaki, Hirano/Kuroki, and Shimokoshi sub-types are each strains of Kato, Irie, Hirano, and Shimokoshi). Dr. Hayasaka from the Institute of Tropical Medicine, Nagasaki University kindly provided the inactivated SFTS Virus (SFTSV) antigen, which had been inoculated with SFTSV on Vero E6 cells, and been inactivated by paraformaldehyde treatment. The avirulent B38 strain of *Francisella tularensis* suspended in 0.5% wet volume of 5 mg/m*l* phosphate buffered formalin (0.01 M, pH 7.2) was used for the tularemia antigen. The antibody titer to each antigen of rickettsia and SFTSV measured immunoglobulin-G antibody titer using an indirect immuno-peroxidase technique according to Suto [16]. A dilution of 1:40 was used as the cutoff value, and a positive antibody was defined as ≥1:40. The tularemia antibody was measured by bacterial agglutination reaction and the rapid slide agglutination test and values of 1:20 or greater were considered positive [14].

The age distribution was 18 to 90 years old (Average 57, median 62, and mode 69). Since most of these blood collections were obtained during a meeting on strategies to counteract acari-borne diseases offered as part of an event to renew hunting licenses, 594 hunters were sampled. Of the remaining samples, 359 people were non-hunters by self-assessment but hunting experience information was not provided by 199 people. At the time of sampling, each participant was in healthy condition with no subjective

Table 2. The antibody titers of 93 positive samples against acari-borne pathogens

Anitibody Titer	*Rickettsia japonica*	*Rickettsia typhi*	*Orientia tsutsugamushi*						SFTSV	*Francisella tularensis* a)
			Gilliam	Karp	Kato	Irie/Kawasaki	Hirano/Kuroi	Shimokoshi		
20										10
40	10	2	4	6	2	nd	4	8	1	1
80	8	1	2	3	2	1	4	7	nd	1
160	13	7	1	1	nd	3	6	2	1	nd
320	7	3	3	2	nd	nd	1	1	nd	nd
640	3	3	nd	nd	nd	2	nd	1	nd	nd
1,280	nd	1	nd	nd	nd	1	1	nd	nd	nd

nd: no detected. Positive antibodies were defined as ≥1:40 except tularemia antibodies. a) The tularemia antibodies of 1:20 or greater were considered positive.

symptoms, but 93 samples were positive against acari-borne pathogens. These antibody titers were distributed around the cut-off level except *R. typhi* (Table 2). In addition, the highest antibody titer was 1,280 in one person in Wakayama for *R. typhi*, and two people in Saga for Irie/Kawasaki or Hirano/Kuroi, which are *O. tsutsugamushi* serotypes.

Numbers of positives for each blood collection method were 15 in 99 serums from the upper arm, 5 of 153 samples by the filter-paper technique, and 73 of 901 samples by Kantan Saiketsu Set. The positive rate of samples by the filter method was lower than other blood collection methods in the chi-square test (P<0.01).

The antibody survey detected positive antibodies from 13 prefectures including Saitama and Hiroshima and other prefectures near the event areas. In Tochigi and Shimane, total blood samples collected exceeded 200. The positive antibody rates of Tochigi (12%, P<0.01) and Nagasaki (16%, P<0.05) were higher compared to other prefectures. A single antibody carrier was detected among 94 people from Fukushima, and the ratio was significantly lower compared with other prefectures (1%, P<0.01). Incidentally, all blood samples in Fukushima were collected using the filter method. We suggested that the filter method is not suitable for this sero survey, and then stopped using it. No positive samples were detected from 26 people in Okinawa. The total positive rate was 8% in this study; there were 51 positive hunters and 29 positive non-hunters in these blood samples that included 594 hunter and 359 non-hunter samples; these results were not significantly different by chi-squared test (P=0.784).

Considering each infectious agent individually, 41 people had anti-*R. japonica* IgG, 17 had anti-*R. typhi* and 35 had Tsutsugamushi diseases (Table 1). Among *O. tsutsugamushi* serotypes, positive samples included 10 Gilliam, 12 Karp, 4 Kato, 7 Irie/Kawasaki, 16 Hirano/Kuroki, and 19 Shimokoshi, and included 17 samples that were positive at two or more serotypes. Moreover, agglutinin as antibody to *F. tularensis,* the cause of tularemia, was detected in 12 people, and two people had anti-SFTSV IgG in Shimane and Wakayama.

The positive antibody prevalence was computed for every classification to evaluate the infection risk among areas inhabited or hunting experiences. The antibody prevalence for a person was counted as one positive even if it was observed against multiple infectious agents. Generalized linear model analysis of the binomial distribution was used to test for significant differences in pathogen positive antibody rates among resident area, age, and gender. The 1,152 people sampled for blood were divided into four classes of rural farm areas: 285 people were from a flat farming area; 110 people were from a hilly farming area; 369 people were from a mountainous farming area; and 388 people were from urban areas. The anti-acari-borne-pathogen antibody titers were 4.9% in urban areas, 9.1% in flat farming areas, 8.1% in hilly farming areas, and 10.1% in mountainous farming areas. Anti-*R. japonica* positives included 4 residents in urban areas, 3 in flat farming areas, 14 in hilly farming areas, and 20 in mountainous farming areas (Fig. 1). Anti-*R. typhi* were 2 residents in urban areas, 2 in flat farming areas, 8 in hilly farming areas, and 5 in mountainous farming areas. Anti-*O. tsutsugamushi* serotypes were 6 positives in urban areas, 4 in flat farming areas, 10 in hilly farming areas, and 15 in mountainous farming areas. Anti-*F. tularensis* included 3 positives in urban areas, 2 in flat farming areas, 2 in hilly farming areas, and 5 in mountainous farming areas. Both mountainous farming area positives were SFTS.

The generalized liner model analysis suggested that the positive rate of anti-*R. japonica* within a resident area was mainly affected by three factors, which were the resident area, age, and gender (Table 3). Age influenced *R. typhi* and Tsutsugamushi diseases. There were three *R. typhi* positives in the senior generation, and Tsutsugamushi disease positives in the younger generation. Rates of anti-*F. tularensis* positives were higher in females than males. For SFTSV, it was not possible to analyze main factors because we found only two positive samples. Another study also reported that the antibody-positive rates for SFTS were low [3].

The questionnaire was implemented since February 2018 and received a total of 307 responses about tick and mite awareness. The results were: 100 residents received no bites, 106 residents were bitten 1–4 times and 101 residents were bitten more than five times. When these data were also divided into four classes of rural farm areas, 69 residents lived in urban areas, 45 residents lived in flat farming areas, 98 residents lived in hilly farming areas and 95 residents lived in mountainous farming areas. These data were evaluated by a residual analysis in the chi-square test (Fig. 2). There was a significantly smaller number of inhabitants who were bitten more than 5 times in urban and flat farming areas. On the other hand, there was a significantly larger number of inhabitants who were bitten more than 5 times in mountainous farming areas. In hilly farming areas, there was a significantly smaller number of residents who had never been bitten.

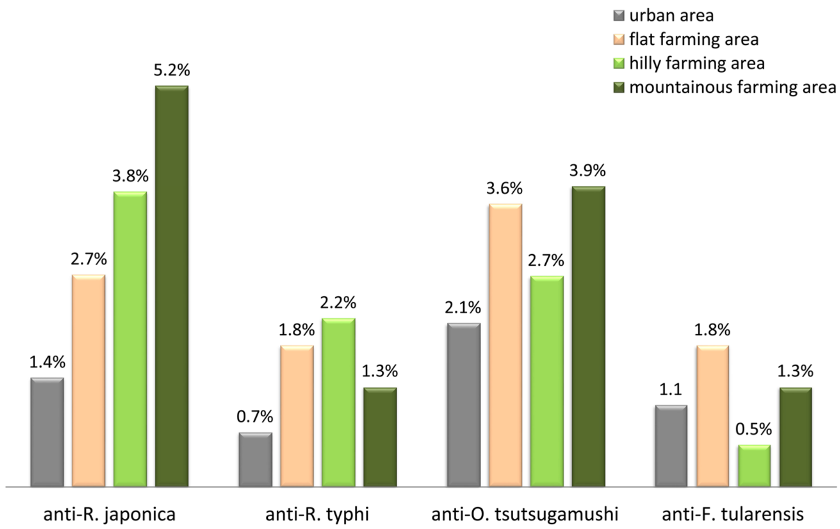

Fig. 1. Rates of positive for anti-acari borne diseases, which are Anti-*R. japonica*, Anti-*R. typhi*, Anti-*O. tsutsugamushi* serotypes, and Anti-*F. tularensis*, in four classes of urban, flat, hilly and mountainous farming areas.

Table 3. Estimated model formulas by the generalized linear model analysis

anti-pathogen	Model formula	AIC	Explanatory variable	Coefficients	Influence for odds (exponential function of coefficients)
Logic (anti-*R. japonica*)	=β1+β2area+β3gender+β4age	356.41	Urban area	–0.664	0.52
	=β1+β2area+β3age	354.61	Flat farming area	0.000	1.00
	=β1+β2area	353.60	Hilly farming area	0.353	1.42
			Mountainous farming area	0.667	1.95
Logic (anti-*R. typhi*)	=β1+β2area+β3genderx+β4age	179.19	Age	0.052	1.05
	=β1+β2gender+β3age	174.86			
	=β1+β2age	173.42			
Logic (anti-*O. tsutsugamushi*)	=β1+β2area+β3genderx+β4age	317.88	Age	–0.017	0.98
	=β1+β2gender+β3age	314.71			
	=β1+β2age	314.08			
Logic (anti-*F. tularensis*)	=β1+β2area+β3gender+β4age	138.52	Gender (male)	–1.184	0.31
	=β1+β2gender+β3age	134.59			
	=β1+β2gender	134.10			

Values are coefficients of the selected model among the resident area, age, and gender and its influence for odds of the positive rate of each anti-acari borne disease.

In this research we tried to evaluate the risk of acari-borne disease among rural residents including, wildlife managers, based on the number of times bitten, and the rate of antibodies in rural areas. We detected positive antibodies for pathogens in all research sites investigated with the exception of Okinawa. Although Tochigi and Nagasaki appeared to have higher rates of positive antibodies, we did not have enough data points to test for local differences among all prefectures. In addition, this study showed that people living in mountainous areas were at a significantly higher risk of being infected with acari-borne diseases, especially Japanese spotted fever or similar antigenic pathogens. Our questionnaire supported the blood tests and indicated that residents were most often bitten by ticks or mites in mountainous areas. Based on the high prevalence of infectious diseases, living and gaining subsistence in the mountains of Japan posed an increased risk of suffering from acari-borne infections.

Currently, the human population in mountainous areas is decreasing as the citizens age, and even places where societies were established have begun to return to the forest with only small populations remaining. The temperate and humid climate promotes vegetative growth, and has changed farming areas back to Japanese pampas fields. Fields and rice paddies that have been abandoned and are no longer cultivated become places for wild boars to obtain food resources, and it is thought that it leads to habitat expansion [5]. Moreover, in recent years, in a city of one million people, such as Kobe and Kyoto, wild boars have been reported to be running in the town [4]. Therefore, it is possible that acari-borne disease risk will increase in flat farming areas and even urban areas.

Given the higher infectious risk in mountainous areas, wildlife managers must be careful to avoid acari bites while accessing forests to reduce mammal populations. Therefore, if it is not possible to educate hunters conducting the wildlife management, there

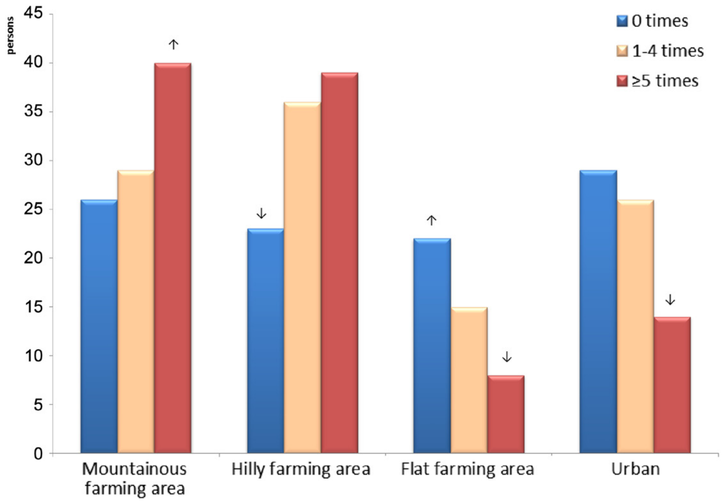

Fig. 2. The proportion of respondents with 0, 1–4, or ≥5 times of tick bites at each region. ↑ or ↓ is indicates significantly higher or lower proportions at a *P*-value <5% by a residual analysis in the chi-square test. There was a significantly smaller number of inhabitants who were bitten ≥5 times in urban and flat farming areas. There was a significantly larger number of inhabitants who were bitten ≥5 times in mountainous farming areas. In hilly farming areas, there was a significantly smaller number of residents who had never been bitten than other areas.

is a risk that they may become victims of infectious diseases.

Infectious risk education is necessary for protection of wildlife managers and hunters but also for general residents in rural areas from a public health perspective. Our study showed that Tsutsugamushi diseases are dangerous, especially to young generations without sufficient knowledge of endemic illness. Therefore, it is especially important to educate people who are new to rural areas on the risk of acari-borne infectious disease.

Incidentally, this project received approval that it "met ethical considerations for research using humans" from Utsunomiya University (registration number H15-0042).

CONFLICT OF INTEREST. The authors declare that they have no competing interests.

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