Seroepidemiologic Survey of *Coxiella burnetii* and Attempt to Detect Coxiella DNA in Aged Non-Laying Chickens in a Prefecture of Japan where Poultry Farming Prosper

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**ABSTRACT.** The indirect fluorescent antibody test (IFAT) revealed seropositivity to *Coxiella burnetii* in aged non-laying chickens in poultry farms in a prefecture in the central part of Japan. Seropositivity was 7%, and antibody titers ranged from 16 to 64. No DNA fragment specific for *C. burnetii* was detected in the chickens by nested-PCR. The prevalence of *C. burnetii* infection in a prefecture in Japan in which poultry farming prospered was 7%.

**KEY WORDS:** chicken, *Coxiella burnetii*, epidemiology.

*Coxiella burnetii* is the causative agent of Q fever in humans and coxiellosis in animals. Q fever is also known as one of the food-borne diseases. In 1959, transovular transmission of *C. burnetii* in chickens was reported [4]. However, it was reported that vertical transmission and ovarian infection of this bacterium was not found in experimentally infected chickens in 1978 [5]. There are few reports on coxiella in chickens and chicken products worldwide. Chicken products have recently been suspected as an infection route of Q fever in humans in Japan [2, 8]. In this study, we surveyed the prevalence of *C. burnetii* infection in aged non-laying chickens in poultry farms in a prefecture in the central part of Japan where poultry farming is one of the most popular industries.

Whole blood samples were collected from 101 aged non-laying chickens in 13 poultry farms (farms A to M) in Aichi Prefecture from July to October in 2003. Aichi Prefecture is located in the central part of Japan facing the Pacific Ocean (from 35° 25′ 18″ N to 34° 34′ 26″ N, and 136° 40′ 26″ E to 137° 50′ 28″ E). The prefecture adjoins 4 prefectures, including Shizuoka, Mie, and Gifu Prefectures. The ages of the chickens ranged from 545 days to 766 days. Each blood sample was centrifuged to separate serum and buffy coat portion except one clotted sample. A total of 100 serum samples were used for a seroepidemiologic survey by the IFAT. The 100 buffy coat samples and 1 blood clot were subjected to DNA extraction, and then the extracted DNA was used for detection of coxiella DNA by nested-PCR. We purified *C. burnetii* Nine Mile strain phase II (American Type Culture Collection VR-616) to utilize as an antigen for the IFAT [1]. The IFAT was performed as previously described with slight modification [3]. We started the IFAT with 4-fold diluted serum samples. A fluorescein-5′-isothiocyanate-conjugated anti-chicken IgG (H+L) rabbit IgG (Organon Teknika, NC) was used as a secondary antibody. A reference chicken serum having an antibody titer of 6400 against *C. burnetii* was used for the IFAT as a positive control. DNA was extracted from theuffy coat samples and the blood clot by a QIAamp DNA Blood Midi Kit (QIAGEN, Tokyo, Japan) in accordance with the manufacturer’s instructions. To amplify the DNA fragment specific for coxiella outer membrane protein, nested-PCR was performed as previously described [7].

The prevalence of IgG antibody against *C. burnetii* in the aged non-laying chickens studied is shown in Fig. 1. We regarded chickens having an antibody titer of ≥16 as positive. Seven (7%) of the 100 serum samples were positive for

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**Fig. 1.** Prevalence of IgG antibody against *C. burnetii* in aged non-laying chicken studied.
the antibody against *C. burnetii*. Five of the 13 poultry farms had positive chickens: 1 (5%) of 20 samples from farm A, 1 (20%) of 5 samples from farm B, 3 (60%) of 5 samples from farm C, 1 (5%) of 20 samples from farm F, and 1 (6.7%) of 15 samples from farm G were seropositive (Table 1). The IgG antibody titers of the 7 samples ranged from 16 to 64 (Table 1). All DNA samples were negative by nested-PCR.

We have provided for the first time seroepidemiologic information on *C. burnetii* in chickens in Aichi Prefecture, Japan. This prefecture is well known as an area where poultry farming prospers. The present study showed that the prevalence and the titer of antibody against *C. burnetii* were low in chickens. Also, the rate of detection of coxiella DNA in chickens was extremely low. Our results indicate that the degree of invasion of coxiella DNA in chickens was extremely low. We therefore cannot compare the present results with results of past studies in Aichi Prefecture. In this study, we suggested that the degree of invasion of coxiella in chickens was low. To obtain detailed information on infection of coxiella in chickens, it is essential to examine chickens in several periods such as the period of introduction of chicks to a farm, egg-breeding period, and aged non-laying period.

In the present study, we surveyed *C. burnetii* infection in chickens in the aged non-laying period. Aged non-laying chickens have the highest risk for infection with *C. burnetii* because they are kept for the longest period in a poultry farm, where the risk of exposure to coxiella from other animals or ticks is high. It is necessary to accumulate more epidemiologic data on *C. burnetii*.

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REFERENCES