Jpn. J. Infect. Dis., 54, 2001

Laboratory and Epidemiology Communications

Detection of Anti-*Coxiella burnetii* Antibodies by Immunofluorescent Technique and Enzyme-Linked Immunosorbent Assay

Yumiko Furuya*, Takashi Katayama, Miyuki Hara, Yoshiya Yoshida, Mitsunobu Imai, Yasutomo Arashima[1], Kimitoshi Kato[1], Motohiko Ogawa[2] and Toshikatsu Hagiwara[2]

Kanagawa Prefectural Public Health Laboratory,
Nakao 1-1-1, Asahi-ku, Yokohama, Kanagawa 241-0815,
[1]Nihon University School of Medicine,
Oyaguchi-Kamimachi 30-1, Itabashi-ku, Tokyo 173-0032 and
[2]National Institute of Infectious Diseases,
Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640

Communicated by Kunihiko Masukawa

(Accepted April 17, 2001)

Q fever is caused by a zoonotic pathogen, *Coxiella burnetii*. Infected humans exhibit influenza-like respiratory symptoms. The antibody-positive population is high among animal farm workers including veterinary doctors (1). It was suggested that some patients diagnosed as having atypical pneumonia or other respiratory infections actually have *C. burnetii* infections (2,3).

This report deals with the detection of anti-*C. burnetii* antibodies among 48 patients with respiratory infections. All the patients showed prolonged fever, cough and respiratory excretion with unknown etiology. Though anti-*C. burnetii* antibody is usually detected by means of the immunofluorescent antibody technique (IF) employing phase II antigen (4), this method requires expertise. Therefore, we performed the IF in combination with ELISA by using phase I and phase II antigens.

Positive control serum from a convalescent Q fever patient (5) showed a titer of ×512 in IF and ×16,000 in ELISA (6) for both phase I and II antigens. *C. burnetii* TK-1 strain was isolated from the patient.

Three among seven sera with IF titers ×16 were negative as determined by ELISA. However, among eight sera with IF titers higher than ×32, only one was negative (Table 1). IF titer ×16 or lower was probably due to non-specific reaction or to cross-reaction. Actually, it has been reported that *Bartonella* or *Legionella* antigens cross-reacted with *C. burnetii* antigen (7,8). Relatively higher titers, >×32, probably reflect specific reactions because the IF and ELISA data matched well.

Positivity tended to be higher for phase II antigen than for phase I antigen (data not shown).

ELISA-positive sera with IF titers higher than ×32 were seen in 7 of 48 (14.6%) respiratory infections (Table 1), while it was seen in 1 of 200 (0.5%) in a healthy population. The positive rate was about 30-fold higher in the respiratory infections. This may indicate the involvement of *C. burnetii* in respiratory infections. However, the involvement of other antigen-cross-reactive pathogens could not be excluded. Further studies with paired sera from more patients will be needed for elucidating the causal relation between *C. burnetii* and chronic respiratory infections.

Table 1. Detection of anti-*C. burnetii* antibody by IF[1] and ELISA[2]

	ELISA (phase II)	
IF titer	negative	positive
<16	30	3
16	3	4
32	1	6
64	0	1
total	34	14

[1]IF was performed by using *C. burnetii* Nine Mile strain phase II cultured in Vero cells as an antigen. The suspension of the cultured *C. burnetii* was spotted on a slide glass. Sera were serially diluted by PBS(–) 2-fold and spotted onto the antigen on the slide glass. After incubation for 1 h, the slide glass was dried, overlaid with FITC-labeled anti-human IgG (Biosource International, Inc., Tago products, Camarille, Calif., USA), incubated for 40 min at 37°C, washed with PBS(–), dried, and then examined using a fluorescent microscope.

[2]ELISA was performed by using phase I and phase II *C. burnetii* Nine Mile strain as antigens (6). The antigens at concentrations of 50 μg/ml were distributed in a volume of 100 μl into each well of 96-well micro-plates (Greiner Labortechnik Ltd., Frickenhausen, Germany) and solidified. Two percent skim milk was used as a blocking reagent. Sera were diluted serially two-fold with PBS(–) containing 0.05% Tween 20 and 0.1% bovine serum albumin (PBS-TB). An aliquot of 100 μl was added to each well. The plates were incubated for 2.5 h at 37°C. After 3 min of washing with PBS-TB three times, peroxidase-labeled anti-human IgG (Biosource International) was added in a volume of 100 μl and incubated for 1 h at 37°C. After 3 min of washing with PBS-TB three times, the color developing agent (1 mg of 3,3',5,5-tetramethylbenzidine, 9 ml of phosphate-citrate buffer, and 2 μl of 30% H_2O_2) was added in a volume of 100 μl and incubated for 15 min at room temperature. The reaction was stopped by adding 50 μl 4N H_2SO_4. The absorbance at 450 nm was measured. An absorbance higher than 2.1-fold or more relative to the negative control was judged as a positive indication.

Phase I and Phase II *C. burnetii* (Nine Mile strain) were obtained from Dr. E. Kovacova, Czechoslovakia Academy of Science.

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*Corresponding author: Tel: +81-45-363-1030, Fax: +81-45-363-1037

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