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Quantitative Assay of *Coxiella burnetii* in Mice

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ABSTRACT

SCHNEIDER, MORRIS D. (IIT Research Institute, Chicago), RICHARD EHRLICH, HERBERT M. YAMASHIROYA, AND SOL MILLER. Quantitative assay of *Coxiella burnetii* in mice. Appl. Microbiol. **14**:767–768. 1966.—Experimental data are presented which demonstrate that the complement-fixing antibody response in individual mice can be used for quantitative assay of *Coxiella burnetii*. The method allows the replacement of a single guinea pig with a single mouse, thus resulting in considerable savings in caging requirements and animal costs.

Various reports have described in vitro and in vivo techniques for quantitation of *Coxiella burnetii*. The in vitro techniques, in which tissue culture (5) or embryonated eggs (4) are used, generally lack the necessary sensitivity. The laboratory mouse has long been known to be susceptible to Q fever infection (1), and has been used in neutralization tests. However, serological titrations in guinea pigs remain as the method of choice for quantitation of *C. burnetii*.

This paper describes a quantitative method for assay of *C. burnetii* in individual Swiss albino mice by the complement-fixation (CF) test after 19 days of incubation. In addition, the incidence of cross infection in mice housed in aggregates was investigated.

MATERIALS AND METHODS

C. burnetii culture. Virulent *C. burnetii* was obtained in the form of an egg yolk sac suspension from the U.S. Army Biological Laboratories, Fort Detrick, Frederick, Md. The culture was stored at solid carbon dioxide temperature until used.

Infectivity titrations. Duplicate 10-fold dilutions of the *C. burnetii* suspension were prepared in sterile phosphate-buffered saline (*p*H 7.7). Just before use, 1,000 units of penicillin was added per ml of the saline. Groups of Swiss albino mice (Webster strain) of either sex, each weighing 15 to 20 g, were inoculated intraperitoneally with 0.1 ml of each duplicate dilution. After injection, the mice were housed in aggregates of 8 or 10 in ventilated cages protected with ultraviolet light.

The mice were maintained on a standard laboratory diet supplemented with fresh greens (cabbage) 3 days before exsanguination to preclude dehydration.

Mice were exsanguinated on the 19th day by snipping the neck vein with a sharp pair of scissors. The blood was collected dropwise directly into disposable plastic tubes (12 by 75 mm). The blood clots were allowed to retract overnight in the refrigerator, and the sera were recovered after low-speed centrifugation.

CF test. Standard qualitative CF tests entailing overnight incubation in a refrigerator were performed in duplicate on the individual sera at a 1:8 dilution. Commercial CF antigen (Markham Laboratories, Chicago, Ill.) prepared from the Nine Mile strain of *C. burnetii* was employed.

Cross-infection studies. To study the incidence of cross infection, mice were injected intraperitoneally with 0.1 ml of *C. burnetii* dilutions or 0.1 ml of the saline diluent. After the injection, the mice were housed in ventilated ultraviolet-protected cages in groups of 8 for 19 or 22 days, at which time they were exsanguinated. The CF test on individual blood sera at 1:8 dilution was employed as the criterion of Q fever infection.

RESULTS AND DISCUSSION

The data obtained from the *C. burnetii* assay in individual mice in four replicate trials entailing eight subsamples, and the results of probit analysis, are shown in Table 1. The 19th day was selected as the optimal time for CF antibody detection on the basis of previously conducted exploratory studies. The mean concentration of infectious *C. burnetii* units in the stock culture was estimated to be $10^{9.2}$ per milliliter. The guinea pig intraperitoneal ID_{50} for the same preparation determined simultaneously was $10^{9.8}$ per milliliter. The difference was not statistically significant.

Analysis of variance (3) was performed on the per cent response data transformed for variance-stabilization with the 2 arcsin $\sqrt{x}$ function (2). The analysis indicated that there were no significant differences between the individual trials or between the subsamples within the trials.

The results of the cross-infection studies were reviewed from the two standpoints: the effect of

TABLE 1. *Coxiella burnetii assay in individual mice*

Trial no.	MIP ID_{50}/ml[a] of stock culture	95% confidence limits	Probit slope
1	9.1	8.9–9.4	1.45
2	9.6	9.2–9.9	1.23
3	9.0	8.6–9.3	0.92
4	9.1	8.7–9.5	0.91
Mean	9.2	8.9–9.5	1.13

[a] Log 10 reciprocal of mouse intraperitoneal ID_{50}.

TABLE 2. *Infection ratio based on Coxiella burnetii dilution*

C. burnetii dilution	CF response (positive/total)			
	19 days		22 days	
	Control	Challenged	Control	Challenged
10^{-1}	3/86	66/69	5/122	112/113
10^{-3}	1/54	67/67	2/93	100/101
10^{-5}	1/41	51/51	2/63	64/64
10^{-7}	0/19	19/21	0/39	36/38

TABLE 3. *Infection ratio based on caging conditions*

Housing ratio		CF response (positive/total)			
		19 days		22 days	
Challenged	Control	Control	Challenged	Control	Challenged
6	2	1/19	63/66	1/34	102/102
5	3	1/32	50/50	0/48	80/82
4	4	0/40	39/41	1/55	56/57
3	5	2/28	28/28	3/72	40/40
2	6	1/33	23/23	4/108	34/35

C. burnetii dilution (Table 2) and the effect of caging ratio (Table 3).

The infection incidence in uninoculated mice based on the dilution factor was very low, with a mean of 2.5 and 2.8% for the 19- and 22-day incubation periods, respectively. The overall incidence of infection in mice challenged with *C. burnetii* was 97.6 and 98.7% for the two incubation periods. It was also shown that the incidence of infection was not related to the ratio of control and challenged mice held together during the incubation period (Table 3). Neither isolation nor demonstration of *C. burnetii* in infected or uninfected mice was attempted.

The possibility of contact transmission of Q fever in the cross-infection studies cannot be ruled out entirely. However, the data indicate that detectable CF antibody from this source did occur only in an insignificant number of mice during the 22-day holding period.

Babudieri (1) reported that mice die easily from infection caused by contaminant bacteria present in the inoculum. The results of our study show that the *C. burnetii* suspension contained initially a concentration of 7.4×10^7 viable bacteria per milliliter. Incorporation of penicillin in the phosphate-buffered saline reduced the count to 4.5×10^6 per milliliter. The presence of the contaminant bacteria did not result in significant mortality and did not interfere with the specific antibody response. Only 4% of the samples were lost, and this resulted mainly from traumatic deaths and harvesting of the blood.

The experimental results demonstrate that the CF antibody response in individual mice is reproducible and can be used successfully for quantitative assay of *C. burnetii*. The method allows the replacement of a single guinea pig with a single mouse. Since the cross-infection studies indicated that mice can be housed in aggregates of eight, without significant incidence of cross infection, considerable savings in caging requirements and animal costs can be expected.

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