Comparison of Enzyme-Linked Immunosorbent Assay and Complement Fixation and Indirect Fluorescent-Antibody Tests for Detection of Coxiella burnetii Antibody

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An enzyme-linked immunosorbent assay (ELISA) was developed to detect immunoglobulin G to Coxiella burnetii phase II. Serum samples from 213 patients who had had Q fever 1 year previously and from 301 blood donors from six localities in Switzerland were tested by ELISA and by indirect fluorescent-antibody (IFA) and complement fixation (CF) tests. The ELISA and the IFA and CF tests detected antibody to C. burnetii in 202 (94.8%), 193 (90.6%), and 166 (77.8%) of the 213 Q fever patients, respectively. With the serum samples from blood donors, the ELISA yielded a higher percentage of positive sera than did the IFA and CF tests. The high specificity of the three tests was confirmed by analyzing paired serum samples from 36 patients suffering from acute pneumonia of viral or bacterial origin. In these cases, the serological results were negative by the three tests, except for three Q-fever cases included as positive control.

Q fever was described for the first time in 1937 in Australia (7). Since then, the worldwide distribution of the disease has been recognized. Coxiella burnetii is an obligate intracellular rickettsial organism, which causes Q fever in animals and humans. This bacterium was named after F. M. Burnet, who discovered the microorganism in the spleens of experimentally infected mice (7), and after H. R. Cox, who succeeded in culturing it in embryonated eggs (5).

Human infections result from contact with infected sheep, goats, or cattle or from infected placentas. Although infections in animals are usually asymptomatic, apart from causing more frequent abortions, the most common clinical presentation in humans is an influenza-like disease, often accompanied by pneumonia (2). However, chronic disease, particularly endocarditis, may appear years after the primary episode (13, 22).

The diagnosis of both primary and chronic Q fever by serology is desirable, since culturing the organism is hazardous and requires specially equipped laboratories.

Unique to C. burnetii is its antigenc-phage phase. The virulent phase I is isolated during natural or laboratory infections of humans or animals, whereas the avirulent phase II develops during serial passage in immunologically incompetent hosts, such as fertilized eggs or cell cultures. This phase transition seems to relate to some of the biological characteristics of the smooth-rough lipopolysaccharide variation (1). Serologically, anti-phase I antibodies are present at high titers only during the chronic form of the illness, whereas anti-phase II antibodies are largely predominant in primary acute Q fever (18).

In 1948 Gsell (12) and in 1952 and 1956 Wiesmann et al. (23, 24) investigated a few outbreaks of Q fever in Switzerland. However, apart from these reports and the one recently published by Bruppacher et al. (3), the incidence of Q fever in Switzerland was not known. In the past, the complement fixation (CF) test was used to detect antibody to C. burnetii. Recently, we have shown that incidence of Q fever may be higher than estimated if the indirect fluorescent-antibody (IFA) test is used rather than the CF test (9).

Since 1983, a few papers have dealt with immunoenzymatic tests for the diagnosis of Q fever (8, 11, 21). In this paper, we describe the enzyme-linked immunosorbent assay (ELISA) and compare it with the CF and IFA tests to detect human immunoglobulin G (IgG) to C. burnetii phase II.

MATERIALS AND METHODS

Serum samples. We received 301 blood samples collected in Switzerland with the Sion, Martigny, Geneva, and Bern blood transfusion centers of the Swiss Red Cross. As controls, 36 paired blood samples were collected in Sion from hospitalized patients suffering from acute pneumonia of viral or bacterial origins (influenza virus, parainfluenza virus, picornavirus, respiratory syncytial virus, Epstein-Barr virus, Chlamydia psittaci, and Mycoplasma pneumoniae); also included were samples taken from three Q fever cases as a positive control. Diagnostic criteria for these infections were a seroconversion or a twofold or higher increase in the titer of CF or IFA tests, or both. Furthermore, we tested 213 blood samples collected in Switzerland from patients who had had Q fever during a large outbreak 1 year previously (10). All these cases were diagnosed by the CF test with the criteria described above and by the detection of specific IgM in the IFA test (19).

ELISA. The ELISA samples were prepared in microtiter plates. Optimal concentrations of antigen, serum, and enzyme conjugate were determined by checkerboard titration. Purified C. burnetii phase II (strain Nine Mile) (Commonwealth Serum Laboratories, Melbourne, Australia) was sonicated for 30 min. Each well of flat-bottom polystyrene microtiter plates was coated with 100 μl of C. burnetii suspension diluted 1:200 in 0.1 M sodium carbonate buffer (pH 9.6). After incubation for 3 h at 37°C, the plates were kept at 4°C until needed. The plates were washed twice by running 400 ml of saline-Tween (0.9% NaCl, 0.05% Tween 20) in the washing device (Virion International, Cham,

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Switzerland). The serum dilutions were prepared in phosphate-buffered saline-Tween buffer (phosphate-buffered saline [pH 7.2], 0.01 M MgCl₂, 0.1% Tween 20) (negative control, 1:50 to 1:1,600; positive control, 1:100 to 1:12,800; test serum, 1:200), and 100 µl of each serum was diluted in all wells, and the samples to be screened were diluted in duplicate, and two wells were left free of serum, one serving as control for the conjugate and the other serving as control for the substrate. The first incubation was done at 37°C for 1 h. The reaction was stopped by addition of 25 µl of 3 N NaOH per well, and the optical density (OD) was read with the Virion reader; the OD of the substrate control well was subtracted. The fifth dilution (1:3,200) of the positive control was calibrated by computer to an OD of 0.35 for each plate. This value served as the cutoff. In this way, we were able to compare results among plates, even if the intensity of colored reactions differed between runs.

IFA and CF tests. The IFA test was performed by the technique described by Philip et al. (20) and adapted to the C. burnetii antigen (phase II, strain Nine Mile; Rocky Mountain Laboratories, Hamilton, Mont.) as described elsewhere (19). The serum samples were screened at a dilution of 1:20. Positive serum samples were then diluted in twofold steps to 1:640.

The serum samples were also tested by the standard CF micromethod (17) with an antigen phase II, strain Nine Mile (Virion International), as described previously (19). Screening with this test was done at a 1:10 dilution. Positive serum samples were titrated twofold to 1:320 dilution.

RESULTS

Reproducibility of ELISA. The OD of the positive control averaged 0.63 ± 0.07 (standard deviation) at a dilution of 1:200. In the same way, the OD of the negative control averaged 0.12 ± 0.05 at a dilution of 1:200.

Sensitivity. Table 1 summarizes the percentages of positive serum samples from the 301 blood donors from different towns in Switzerland. They vary from 16.7 to 35.7%, from 13.3 to 33.3%, and from 6.7 to 28.6% as detected by ELISA, IFA, and CF, respectively. The ELISA revealed 86 positive samples of 301 tested (28.6%), whereas IFA detected 68 (22.6%) and CF detected 53 (17.6%). The results of ELISA and CF are not statistically different (x² = 3.2; 1 df) by the χ² method. It should be pointed out that 10 and 11 serum samples from Romanshorn and Zollbrück, respectively, were anticomplementary in the CF test. The ELISA and IFA (Fig. 1), as well as the ELISA and CF (Fig. 2), were compared for 140 serum samples from Leytron and Sion together (Table 1). The ELISA revealed 43 positive samples, of which 9 were negative by IFA (Fig. 1). Two samples found positive by IFA were negative by ELISA. Of the same 43 ELISA-positive samples, 19 were negative by CF, and 3 CF-positive samples were negative by ELISA (Fig. 2).

Of the 213 patients having had Q fever 1 year previously (Fig. 3 and 4), 202 (94.8%) still had antibody to C. burnetii detectable by ELISA, 193 (90.6%) had antibody detectable by IFA, and 166 (77.8%) had antibody detectable by CF. All the CF-positive serum samples were detected by ELISA, whereas three IFA-positive samples were negative by ELISA. For this group of patients, the χ² method shows that the results of ELISA and CF (χ² = 5.12; 1 df), but not those of ELISA and IFA (χ² = 1.68; 1 df), are statistically different. The results of IFA and CF (χ² = 3.63; 1 df) were not significantly different.

Of the 514 tested serum samples, 302 were found positive by at least one test (Table 2). Only one serum sample which was positive by the IFA and CF tests was negative by ELISA. In the same way, 13 samples that were negative by ELISA were positive by IFA or CF. Thus the ELISA detected 288 of a total of 302 positive serum samples (95.4%). For comparison, IFA and CF revealed 260 (86.1%) and 219 (72.5%) positive samples. The number positive by

| TABLE 1. CF, IFA, and ELISA IgG to C. burnetii phase II in blood donors |
|------------------------|------------------------|------------------------|
| Locality (canton)*     | No. of samples tested | No. (% of samples positive by: |
|                       |                        | ELISA                   | IFA                   | CF         |
| Geneva (GE)°           | 59                     | 14 (23.7)               | 9 (15.3)              | 8 (13.6)   |
| Romanshorn (TG)        | 30                     | 9 (30.0)                | 5 (16.7)              | 2*         |
| Zollbrück (BE)         | 35                     | 5 (16.7)                | 4 (13.3)              | 4*         |
| Sembrancher (VS)       | 42                     | 15 (35.7)               | 14 (33.3)             | 12 (28.6)  |
| Leytron (VS)           | 70                     | 23 (32.9)               | 22 (31.4)             | 16 (22.2)  |
| Sion (VS)°             | 70                     | 20 (28.6)               | 14 (20.0)             | 11 (15.7)  |
| Total                  | 86 (28.6)              | 68 (22.6)               | 53 (17.6)             |

* GE, Geneva; TG, Thurgau; BE, Bern; VS, Valais.
° Surrounding towns are included.
° A total of 30% of the sera were anticomplementary.
the three tests corresponded to 69.2% of all the positive serum samples (209 of 302), and 84.8% (256 of 302) of the samples positive by ELISA were confirmed by IFA or CF. Furthermore, there was a good correlation between increasing ELISA OD values and increasing IFA titers. The correlation was not as good between the ELISA OD values and CF titers, above all for samples with high CF antibody titers (Fig. 4).

**Specificity.** The paired serum samples from the three patients suffering from Q fever, which were included as a control, showed a seroconversion, the OD values varying from 0.07 to 0.60 and from 0.16 to 1.06 for the first two cases and showing a high increase for the third (0.41 to 0.92).

On the other hand, no significant OD variations were observed between the paired serum samples from patients with acute pneumonia of other origin. However, the OD value of two paired serum samples varied from 0.33 to 0.38 (negative to positive) and the other varied from 0.36 to 0.28 (positive to negative); however, all four samples showed OD values close to the cutoff value of 0.35 ± 0.06 (standard deviation). Similarly, we did not observe any significant variation of the titers with the IFA and CF tests, except for the three acute Q fever cases.

**DISCUSSION**

Our findings suggest that the sensitivity of the ELISA is superior to those of the IFA and CF tests. Indeed, the ELISA detected more positive serum samples from patients who had had Q fever 1 year previously (10) than did the IFA or CF test. At 1 year after the acute episode, no antibody was detected by CF in 22.2% of the patients, by IFA in 9.4% of the patients, and by ELISA in only 5.2% of the patients. Statistical analysis of the results by the χ² method showed that the difference, between the ELISA and the CF test only,
is significant. Similar results were observed with blood donors, although the differences were not significant. Such differences between ELISA and the other tests were not surprising, because of the higher sensitivity of immunoenzymatic tests over CF or IFA for Q fever. This has also been reported by Krauss et al. (15) and Rogers and Edlinger (21), and it is known to occur with other rickettsiae (4, 6, 14). In our study, the percentage of positive serum samples among blood donors is not representative of the Swiss population, since most of the donors came from rural localities. Nevertheless, our results show that cases of Q fever are more frequent than has been generally assumed.

The various serologic tests used for Q fever (CF, IFA, microagglutination, hemagglutination, etc.) are highly specific, and there are no known cross-reactions between C. burnetii and any bacterial or viral microorganisms (16). The limited study with our ELISA confirmed this specificity, since no significant OD variations were observed in serum samples from patients suffering from pneumonia of other origin. The same observations were made with IFA as the routine diagnostic test during the past 3 years in our laboratory.

At least 50% of the positive serum samples from blood donors had titers of 1:10 (threshold) by CF. An increase or decrease of titer of 1 dilution in this test could halve or double the number of positive serum samples, depending on the sensitivity of the test. For IFA and ELISA, on the other hand, titers or OD values were spread over a larger scale and only a few values were close to the cutoff. Another problem, encountered with CF, is anticomplementary activity as recorded with serum samples from Romanshorn and Zollikon (Table 1). Such reactions seem to be related to the nature as well as the storage conditions of the serum samples. Contaminated samples used in the ELISA may give false-positive reactions, whereas they will not influence the IFA results. Although the IFA is simple to perform and very economical in the use of reagents, reading is subjective and is tiring when a large number of sera are to be screened.

Our results indicate that for epidemiological survey, the ELISA is the test of choice. It is simple to perform and is more sensitive than the other tests used. The ELISA is also useful for the diagnosis of acute cases, as reported by Döller et al. (8) and Field et al. (11). At present, we are evaluating the ELISA for the diagnosis of acute and chronic Q fever by monitoring class-specific IgG, IgM, and IgA to phases I and II of C. burnetii.

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LITERATURE CITED


