Animals with *Coxiella burnetii* infection demonstrate a Western immunoblot profile of chronic Q fever in humans

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TJ MARRIE, L YATES. Animals with *Coxiella burnetii* infection demonstrate a Western immunoblot profile of chronic Q fever in humans. *Can J Infect Dis* 1996;7(1):45-48. Western immunoblotting was used to compare the immune response to *Coxiella burnetii* phase I and phase II antigens of humans with acute and chronic Q fever with that of infected cats, rabbits, cows and raccoons. The cats, rabbits, cows and raccoons had an immunoblot profile similar to that of the human with chronic Q fever.

Key Words: *Coxiella burnetii*, Q fever

Des animaux porteurs d'une infection à *Coxiella burnetii* manifestent le même profil que des humains atteints de fièvre Q chronique au test Western immunoblot

RÉSUMÉ : L'immuno-empreinte de type Western immunoblot a été utilisée pour comparer la réponse immunitaire aux antigènes de phases I et II de *Coxiella burnetii* d'humains atteints de fièvre Q aiguë et chronique, à celles de chats, de lapins, de vaches et de rats-laveurs infectés. Les chats, les lapins, les vaches et les rats-laveurs ont manifesté un profil d'immuno-empreinte semblable à celui d'humains atteints de fièvre Q.

Q fever is a zoonosis that results from infection with the microorganism *Coxiella burnetii* (1,2). While there is an extensive wildlife reservoir of *C. burnetii* (2), cattle, sheep and goats are the domestic animals that are usually responsible for the spread of this organism to humans (1). The epidemiology of Q fever in Nova Scotia is unique in that infected parturient cats have been implicated in the spread of this infection to humans in this province (3).

Q fever in humans causes both acute and chronic infections (1). The former includes a self-limited febrile illness, pneumonia or hepatitis. The latter almost always is endocarditis or other intravascular infection and rarely osteomyelitis (1).

The objective of this study was to examine the immune response of a variety of animals to *C. burnetii* phase I and phase II antigens by using Western immunoblotting.

MATERIALS AND METHODS

Serum samples: Serum samples from eight humans with acute and four with chronic Q fever and from 14 seropositive cats, eight rabbits, eight raccoons, three cows and one dog were used.

Determination of antibody titres to phase I and phase II *C. burnetii* antigens: Antibody titres to phase I and phase II

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TABLE 1
Antibody titres to phase I and phase II *Coxiella burnetii* antigens, by indirect immunofluorescence antibody test, of the sera from the various animals

Animal	Phase I	Phase II
Human		
Acute Q fever		
Acute phase	1:256	1:4096
Convalescent phase	1:128	1:4096
Chronic Q fever	1:65,536	1:8192
Raccoon	1:32	< 1:8
Dog	1:256	1:8192
Cow	> 1:4096	1:1024
Rabbit	1:512	1:64
Cat	1:64	1:512

C burnetii antigens were determined using a microimmunofluorescence test as previously described (4). Anticat, antirabbit, antibovine and antihuman fluorescein isothiocyanate-conjugated antisera were obtained from Data Immunoglobulins (Dakopatts, Glostrup, Denmark). Antiraccoon fluorescein isothiocyanate-conjugated antiserum was obtained from Zymed Laboratories (California).

An antibody titre of 1:8 or greater to either phase I or phase II *C burnetii* antigen was considered a positive result. Sera were titred to end-point.

Western immunoblotting: *C burnetii* phase I (CB9M1C7) and phase II (CB9M2C4) whole cells were gamma-irradiated at -70°C with 1.5 to 2 million rads and diluted to 1 mg/mL aliquots in phosphate buffered saline. Before use, samples were thawed and centrifuged for 10 mins. The supernatant was discarded and the pellet was resuspended in 1 mL of Laemmli sample buffer (5) and boiled for 5 mins. One milligram of whole-cell antigen was used in a volume of 1 mL for each set of sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Molecular mass markers ranging from 200 kDa to 14.3 kDa were prestained (Sigma, Missouri) and were prepared according to the manufacturer's instructions. In addition, *Legionella pneumophila* heat shock protein, molecular mass 58 kDa (6), which has homology to *C burnetii* heat shock protein, was obtained and was used to identify the antibodies reacting with the *C burnetii* heat shock protein.

Electrophoresis: Gels were formed in a Biomed Protein II gel electrophoresis unit (Bio Rad). The gel running buffer consisted of 0.3% Tris (Sigma), 1.44% glycine (Sigma) and 0.1% SDS. The pH was adjusted to 8.3. Electrophoresis was carried out for 4.5 to 6.5 h at 41 to 51 mA. The electrophoresis apparatus was kept cool with running water.

Prepared *C burnetii* cells and markers were electrophoresed through a 5% stacking gel before separation in a 12.5% polyacrylamide separation gel. Phase I and phase II *C burnetii* cells were run simultaneously on different gels.

Electrophoretic transfer of proteins to nitrocellulose: Gels were removed from the electrophoresis chamber and equili-

brated along with nitrocellulose paper (NCP) in transfer buffer for 30 mins. The transfer buffer, which contained 0.3% Tris and 1.44% glycine, was adjusted to pH 8.3. Electrophoretic transfer was carried out for 16 h at 30 V.

Western immunoblotting: NCP containing transferred proteins was rocked for 10 mins in buffer containing 50 mM Tris, pH 7.4, and 250 mM sodium chloride. The NCP was blocked for 1.5 h in this buffer with 20% bovine serum albumin (BSA) (Sigma) pH 7, then washed three times at room temperature (21°C) in a buffer consisting of 150 mM sodium chloride, 5 mM EDTA, 50 mM Tris, 0.25% BSA and 0.5% Nonidet P40 (Sigma). Primary antibody was the animal serum diluted in incubation buffer (wash buffer plus 2% BSA). The diluted serum was added to NCP and rocked for 2 h at room temperature. The NCP was then washed five times with a final wash in wash buffer plus 2% BSA for 5 mins and alkaline phosphatase conjugated goat antihuman (anticat, etc). Immunoglobulin (Ig) G was diluted in incubation buffer, added to NCP and rocked for 1 h at room temperature. The NCP was then washed as described above. Ig bound to protein bands was detected by the Biomed alkaline phosphatase conjugate substrate kit as per manufacturer directions. The reaction was stopped after 1 h by two washings in triple distilled water.

RESULTS

Serum samples from eight humans with acute Q fever (four with chronic Q fever), 14 seropositive cats associated with outbreaks of Q fever in humans (3), eight raccoons, one dog, three cows and eight rabbits were examined. All had positive antibody titres to *C burnetii* antigens by the indirect immunofluorescence test – data for the serum samples shown in the figures are given in Table 1. These are representative of the antibody titres for the various animals studied. Figures 1 and 2 are representative of the results and show the immune response of the various animals to *C burnetii* antigens.

All the animal samples tested showed an almost identical response to that of the human with chronic Q fever. The cat seemed to have identical responses to phase II and phase I (Figure 1) antigens. For the remaining animals the phase I response was dominant. The sample from human with chronic Q fever recognized fewer phase II than phase I antigens. The intensity of the bands in Western blot varied considerably for the different animals tested and, except for the raccoon serum, did not correlate with the antibody titre. In contrast, the serum from the human with acute Q fever had antibodies against the 58 kDa heat shock protein only. The cat sample recognized more phase II antigens than did the human with chronic Q fever.

DISCUSSION

In a previous study (7) we found that patients with chronic Q fever recognized eight to 15 *C burnetii* phase I antigens. In contrast, samples from subjects with acute Q fever recognized only four phase I antigens. In addition several antigens were recognized only by sera from patients with chronic Q fever – these included antigens with molecular masses of 50, 80 and 160 kDa.

Figure 1) Western immunoblots showing immunoglobulin G antibodies directed against various antigens of phase I *Coxiella burnetii* cells. The first lane shows the prestained molecular mass markers; hsp is the 58 kDa *Legionella pneumophila* heat shock protein; Acute and conv refer to serum samples from a patient with pneumonia due to *C burnetii*; chr refers to serum from a patient with chronic Q fever; rac raccoon serum; bov bovine serum; rab rabbit serum. The serum sample from the patient with chronic Q fever was tested at a dilution of 1:500, rabbit and cat sera at 1:100 and all others at 1:50

The major finding in the present study is that cats, rabbits, raccoons and cows with antibodies to phase I antigens recognize most of the same antigens as patients with chronic Q fever.

Novak et al (8) analyzed serum samples from mice infected intraperitoneally with *C burnetii* phase I cells. IgG antibodies to 60, 49, and 27 kDa proteins appeared on day 10 after infection, followed by those to 77 kDa protein from day 18 postinfection. Antibodies to seven other proteins of 42 to 70 kDa molecular mass appeared after 28 days of infection. This profile from day 29 onwards is remarkably similar to our findings for rabbits, cats, raccoons and cows.

Willems et al (9) used unfixed *C burnetii* phase I cells and scanned the immunoblots with a laser densitometer. They found that mice three weeks after infection recognized antigens with molecular masses of 13.3, 31.8, 41.7, 44, 46, 50.4 and 52 kDa. The main peaks were at 41.7, 50.4 and 52.5 kDa. An infected cow recognized antigens with molecular masses of 13.9, 23, 30, 57.3 kDa. The one human studied recognized antigens with molecular masses of 14.6, 23, 29.6, 46.5, 59.1 kDa.

The dominant antigen recognized by humans, rabbits, cats, cows and raccoons in our study was the 58 kDa heat shock protein. Heat shock proteins appear in response to a variety of

Figure 2) Western immunoblots showing immunoglobulin G antibodies directed against various antigens of phase II *Coxiella burnetii* cells. The first lane shows the prestained molecular mass markers; hsp is the 58 kDa *Legionella pneumophila* heat shock protein; Acute and conv refer to serum samples from a patient with pneumonia due to *C burnetii*; chr refers to serum from a patient with chronic Q fever; rac raccoon serum; bov bovine serum; rab rabbit serum. The serum sample from the patient with chronic Q fever was tested at a dilution of 1:500, rabbit and cat sera at 1:100 and all others at 1:50

stresses, including temperature elevation, and range in size from 10 to 100 kDa (10,11). The heat shock protein of *C burnetii* has homology with heat shock proteins of 58 to 65 kDa in *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *L pneumophila*, *Pseudomonas aeruginosa*, *Borrelia burgdorferi*, and *Treponema pallidum* (12). Thompson et al (12) found that the major heat shock protein of *C burnetii* had an apparent molecular mass of 62 kDa.

Chronic Q fever has not been recognized in animals other than humans; however, uterine infection and recrudescence of Q fever infection during pregnancy are common in animals other than humans (2). In this regard it is interesting that a human female with primary Q fever during pregnancy who had *C burnetii* isolated from the placenta at parturition had a profile like that of chronic Q fever (unpublished data). It is possible that chronic endometrial infection may be one reason for the predominance of phase I antibodies and the chronic Q fever-like profile on immunoblotting in animals other than humans. The cats, dog and cow were all female, and all had

been associated with Q fever in humans. One of the cats had chronic endometritis as shown by prolonged uterine bleeding following parturition. Lang (13) states that chronic infection by *C. burnetii* of adult animals (other than humans) does not have a cardiac or hepatic localization; instead, the uterus and the mammary glands are sites for chronic shedding of the microorganisms. It is interesting that the cat serum recognized more phase II antigens than did serum from the human with

chronic Q fever. The sex of the rabbits and raccoons was not recorded when blood samples were collected.

It is also possible that animals other than humans have Q fever endocarditis, but it is unlikely that all seropositive animals have this disease, as the immunoblots suggest. Recently we examined the hearts of 20 seropositive raccoons and did not see any vegetations on their valves (unpublished data).

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