Differentiation of *Coxiella burnetii* isolates by analysis of restriction-endonuclease-digested DNA separated by SDS-PAGE

LAURA R. HENDRIX, JAMES E. SAMUEL† AND LOUIS P. MALLAVIA*

Department of Microbiology, Washington State University, Pullman, Washington 99164-4233, USA

(Received 29 May 1990; revised 23 August 1990; accepted 25 September 1990)

Thirty-two isolates of *Coxiella burnetii* collected from various hosts ranging from arthropods to man were compared by restriction endonuclease (RE) digestion patterns of chromosomal DNA using SDS-PAGE. SDS-PAGE provided better DNA fragment separation than agarose gel electrophoresis and enabled the differentiation of these isolates into six distinct groups on the basis of DNA restriction fingerprints. Two groups of chronic disease isolates could be distinguished, each having unique RE digestion patterns of chromosomal DNA. Three similar but distinct RE digestion patterns were seen among the group of acute disease isolates. Three additional isolates included in this study exhibited a unique RE digestion pattern and also had a unique plasmid type, designated QpDG. DNA–DNA hybridization on selected isolates quantified the relatedness between several groups and supported the classification of these groups as distinct strains.

Introduction

Coxiella burnetii, a rickettsial agent, causes a wide spectrum of human disease. Q fever is an acute flu-like illness which is self-limiting or easily treated with antibiotics. Other serious and more difficult-to-treat conditions which can result from infection include pneumonia, hepatitis and chronic endocarditis. The organism has also been implicated as the causative agent of late-term abortion in goats and has been isolated from an extremely wide range of animals and arthropods (Baca & Paretsky, 1983). Despite this wide range of both disease and host, conventional serological methods have failed to discriminate between *C. burnetii* isolates (Topping *et al.*, 1946; Peacock *et al.*, 1983). Recent studies have indicated that *C. burnetii* isolates can be differentiated by several parameters, including the presence of unique plasmid sequences and lipopolysaccharide (LPS) differences. Isolates contained one of two plasmid types or were plasmidless (Samuel *et al.*, 1985; Vodkin *et al.*, 1986). Polyacrylamide gel electrophoresis (PAGE) showed that LPS banding patterns also varied among isolates (Hackstadt, 1986). Both analyses allow the differentiation of isolates into at least three major groups (Samuel *et al.*, 1985). These groups do not

correlate with host, geographical location, or date of isolation. However, the type of human disease caused by isolates in each group is similar: two groups contain isolates from chronic endocarditis cases and no acute disease isolates, while another group contains acute disease isolates and no isolates from humans with chronic disease. This implies that specific isolate groups have a particular virulence potential in humans.

A previous report that compared the banding patterns of *C. burnetii* chromosomal DNA digested by restriction endonucleases (REs) and separated by agarose gel electrophoresis was unable to identify any differences that were consistently associated with phase variation (O'Rourke *et al.*, 1985). To more clearly understand the degree of relatedness between isolate groups, in the present work we used SDS-PAGE separation of RE-digested DNA, which provides much better resolution than electrophoresis on agarose gels, to compare chromosomal DNA from each isolate. DNA–DNA hybridization analysis on selected isolates was also performed to quantify the relatedness between groups. A preliminary report of these data has been previously presented (Mallavia *et al.*, 1985).

Methods

Isolates. Each isolate in this study came from an animal infection or was derived by long-term passage (animal, tissue culture or egg, or combinations thereof) of a clinical isolate and was subsequently

† Present address: BioCarb Inc, Gaithersburg, Maryland 20879, USA.

Abbreviation: RE, restriction endonuclease.

defined as an independent isolate. The isolates were obtained from M. G. Peacock, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT, USA, or were cultured at the Washington State University Biocontainment Laboratory (H WSU 101). Name, catalogue number, origin, and passage history for each isolate are shown in Table 1. Most of these isolates have been described previously (Samuel *et al.*, 1985); they include representatives from the diversity of warm-blooded animals and arthropods from which *C. burnetii* has been recovered. The human isolates were from acute Q fever infections, endocarditis cases, and one case of endocarditis subsequently recategorized as hepatitis (Peacock *et al.*, 1983). Animal isolates were derived from one sheep abortion and four goat abortions, three wild rodents, and the milk of three persistently infected cows. Several isolates (including Nine Mile RSA493) were obtained from ticks. One human isolate, American Q Dyer, is thought to have resulted from a laboratory-acquired infection with the Nine Mile RSA493 isolate (Topping *et al.*, 1946). The passage history refers to the subculturing of each isolate prior to propagation by our laboratory. The phase character of each isolate refers to reactivity with phase I or phase II hyperimmune serum in a complement block titration assay (Peacock *et al.*, 1983).

Propagation and purification of rickettsiae. Suspensions of infected yolk sacs or guinea-pig spleens which had been stored at -80°C were inoculated into 6-d-old, embryonated chick eggs (H and N Hatchery, Redmond, WA, USA). Infected yolk sacs of viable embryos were harvested when significant numbers ($>20\%$) of embryos died, or 2–3 d before hatching if death did not occur. Rickettsiae were purified from the infected yolk sacs as described previously (Hendrix & Mallavia, 1984).

DNA extraction. Purified *C. burnetii* organisms (up to 20 mg dry weight) were suspended in 20 mM-Tris/HCl, 50 mM-NaCl (pH 9.1) and lysed with thermolysin and SDS as reported previously (Samuel *et al.*, 1983). Lysates were centrifuged to equilibrium on caesium chloride gradients with ethidium bromide (CsCl/EtBr) at 200 000 *g* for 16 h in a Beckman Vti80 rotor. DNA bands were located with UV light and the upper chromosomal band collected. The EtBr was removed by extraction with $1 \times \text{SSC}$ (0.15 M-NaCl, 0.015 M-sodium citrate, pH 7)-saturated 2-propanol and the DNA precipitated in 2-propanol at -20°C overnight. The DNA was pelleted by centrifugation at 10 000 *g* and extracted twice in 1:1 (v/v) buffered phenol/chloroform (Maniatis *et al.*, 1982). The DNA was then precipitated in absolute ethanol containing 0.3 M-ammonium acetate at -20°C for 2 h, pelleted, washed with 95% (v/v) ethanol and resuspended in a small volume of 10 mM-Tris/HCl, 1 mM-EDTA (pH 7.4). Although isolated chromosomal DNA was used in these studies, it was recognized that nicked plasmid DNA was also present in these preparations.

Restriction endonuclease treatment and electrophoresis. Restriction enzymes were obtained from New England Biolabs or Bethesda Research Laboratories. DNA was digested with approximately 5 U enzyme ($\mu\text{g DNA}$) $^{-1}$ for 2 h using conditions recommended by the manufacturer. RNAase A ($10 \mu\text{g ml}^{-1}$) was added to each DNA sample before digestion. The DNA was extracted once after digestion with an equal volume of phenol/chloroform to remove restriction enzymes, which also stain with silver stain and can obscure the DNA fragment bands.

SDS-PAGE was carried out under the conditions described by Laemmli (1970). Gels were $160 \times 320 \times 1.5$ mm and were run at 7.5 mA for approximately 24 h with constant cooling. The discontinuous gels consisted of a 7.5% (w/v) separating gel with a 15 mm deep stacking gel of 3% (w/v) polyacrylamide. Each well was loaded with 1–3 μg of restriction-enzyme-digested DNA in Laemmli sample buffer. Gels were stained with silver by the method described by Herring *et al.* (1982) with the modification of McClenaghan *et al.* (1984) to remove

SDS and glycine. Gels were photographed on a light box using an 80B Wratten filter and Polaroid type 55 film.

Probe preparation and Southern blot hybridization. Nick-translation and Southern blot hybridization were performed as previously described (Samuel *et al.*, 1985). The probe consisted of nick-translated plasmid QpH1 DNA. Blots were hybridized at 68°C in $5 \times \text{SSC}$, $10 \times \text{Denhardt's solution}$ ($1 \times \text{Denhardt's solution}$ is 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone), 50 mM-Tris/HCl, pH 8.0, 5 mM-EDTA and washed for 30 min, four times, with $1 \times \text{SSC}$ at 68°C to provide stringent conditions.

DNA solution hybridization. The similarity of chromosomal *C. burnetii* DNA from various isolates was measured by DNA hybridization as described by deLay (1970) and specifically adapted as follows. Purified rickettsiae were lysed with thermolysin and 1% (w/v) SDS and the chromosomal DNA purified as above. The DNA was then treated with phenol/chloroform followed by chloroform extraction and dialysed against $2 \times \text{SSC}$ overnight. The DNA solution, adjusted to an A_{260} of 4.0, was sonicated for 2 min at 30% power with a Fisher sonicator to generate randomly cleaved DNA fragments 600 ± 300 bp in length. The solution of fragmented DNA was then used at an A_{260} of 1.0 in hybridizations with each isolate group. Thermal renaturations were done on a Beckman DU-8 spectrophotometer with T_m software (Beckman Instruments) and temperature-controlled cuvettes with $2 \times \text{SSC}$ as a blank. Samples were raised to 10°C above the T_m as calculated by the formula $T_m = 0.41 (\text{mol\% G + C}) + 69 + 13.3 \log [\text{Na}]$. For *C. burnetii* DNA, the T_m is 81.6°C . The temperature was quickly (<2 min) reduced to the optimal renaturation temperature as calculated by $T_{or} = 1.24T_m - 38.8$. The calculated value for *C. burnetii* DNA is 73°C . The rate of change of $A_{260} \text{ min}^{-1}$ was determined over a 30 min period. The degree of hybridization (D) between DNA samples from two isolates was determined by the formula $D = 4V_m - (V_A + V_B)/2$, where V_m is the decrease in A_{260} of the mixture of DNA samples from both isolates, V_A is the decrease in A_{260} of the DNA sample from one isolate, and V_B is the decrease in A_{260} of the DNA sample from the second isolate.

Results

RE-digested chromosomal DNA separated by SDS-PAGE

The banding patterns of RE-generated DNA fragments of chromosomal DNA allowed each *C. burnetii* isolate to be placed into one of six groups. The resolution of the DNA fragments by agarose gel electrophoresis was not sufficient to allow a close comparison of the RE banding patterns between isolates. For this reason, a procedure using long-gel (320 mm) SDS-PAGE and silver staining similar to the technique reported by McClenaghan *et al.* (1984) for *Chlamydia psittaci* was used. The result was a dramatic increase in resolution of the DNA fragments and, therefore, an increase in the sensitivity of comparison. Fig. 1 shows the results obtained after digestion of DNA from several isolates with *EcoRI* and separation of the fragments on SDS-PAGE. The DNA of isolates that had similar fragment patterns after digestion with *BamHI* (Fig. 2) also exhibited similar patterns when digested with *EcoRI* (Fig. 1). The groupings did not

Fig. 1. *EcoRI* restriction endonuclease digestion of the DNA of twelve *C. burnetii* isolates. Chromosomal DNA was digested with *EcoRI* and fragments separated by SDS-PAGE on a 7.5% gel using Laemmli buffers. DNA bands were visualized by silver staining. Lanes: (A) *HindIII*-digested λ phage and *HaeIII*-digested ϕ X174 phage (size standards); (B) MSU Goat Q177; (C) K Q154; (D) F Q228; (E) P Q173; (F) Canada Goat Q218; (G) Nine Mile RSA493 phase I; (H) Goat Q195; (I) M-44 RSA459; (J) Henzerling RSA331; (K) L Q216; (L) G Q212; (M) S Q217.

Fig. 2. *BamHI* restriction endonuclease digestion of the DNA of twelve *C. burnetii* isolates. Chromosomal DNA was digested with *BamHI* and fragments separated by SDS-PAGE as described for Fig. 1. Lanes: (A) MSU Goat Q177; (B) K Q154; (C) F Q228; (D) P Q173; (E) Canada Goat Q218; (F) Nine Mile RSA493 phase I; (G) Goat Q195; (H) M-44 RSA459; (I) Henzerling RSA331; (J) L Q216; (K) G Q212; (L) S Q217; (M) *HindIII*-digested λ phage and *HaeIII*-digested ϕ X174 phage (size standards).

Fig. 3. *EcoRI* restriction endonuclease digestion of the DNA of passage history variants of six *C. burnetii* isolates. Chromosomal DNA was digested with *EcoRI* and fragments separated by SDS-PAGE as described for Fig. 1. Passage history of each isolate is given in Table 1. Lanes: (A) *HindIII*-digested λ phage and *HaeIII*-digested ϕ X174 phage (size standards); (B) M-44 Q141; (C) M-44 RSA459; (D) American Q Dyer Strain; (E) Dyer RSA345; (F) Ohio RSA270; (G) Ohio RSA338; (H) African RSA334; (I) Giroud RSA431; (J) Nine Mile RSA493 phase I; (K) Nine Mile RSA439 phase II; (L) Nine Mile RSA514; (M) Nine Mile RSA258-A.

change when the DNA samples were digested with *HindIII* (data not shown). Minor differences did occur within a group. For example, the DNA of several of the isolates in genomic group I [El Tayeb, Panama, California 33 and California 16, and Ohio 314 (both RSA270 and RSA330)] had an extra *BamHI* fragment of approximately 4 kb compared to the other isolates in that group, but appeared identical when digested with *EcoRI* (data not shown). The differences were more easily seen when the rickettsial DNA was digested with *BamHI* (Fig. 2) because fewer bands were produced in the size range best resolved by SDS-PAGE.

Effect of passage history on restriction site polymorphism

Fig. 3 shows *EcoRI*-digested DNA from a number of isolates which differed from at least one other isolate on the gel only in terms of passage history. No apparent differences in RE band patterns between the passage-history variants could be seen. One specific banding

difference between *HaeIII*-digested DNA of Nine Mile phase variants using agarose gel electrophoresis has been reported previously (O'Rourke *et al.*, 1985).

Correlation of genomic grouping and plasmid type

On the basis of similar or identical banding patterns of RE-digested chromosomal DNA, the isolates have been placed into six groups (Table 1). This table also identifies the particular type of plasmid DNA present in each isolate. Isolates in chromosomal groups I, II and III have plasmid QpH1, and isolates in group IV have plasmid QpRS. Group V isolates are plasmidless, but have chromosomal DNA sequences showing homology to the *C. burnetii* plasmids. Group VI isolates have a plasmid type not previously reported, termed QpDG. A detailed mapping of all three *C. burnetii* plasmids will be described elsewhere. Figure 4(a) is an autoradiograph of a Southern hybridization showing the sequences homologous to plasmid QpH1 found in each genomic group

Table 1. Isolates of *C. burnetii*

Genomic group*	Plasmid type†	Isolate‡	Phase§	Original source	Disease	Passage
I	QpH1	<i>Nine Mile RSA493</i>	I	Montana, tick, 1935	—	307GP/1TC/1EP
		Nine mile RSA439¶	II	Montana, tick, 1935	—	90EP/1TC/1EP
		Nine mile RSA514¶	I/II#	Montana, tick, 1935	—	4EP/306GP/3EP
		Nine mile RSA285-A¶	I/II#	Montana, tick, 1935	—	4EP/306GP/3EP
		Dyer RSA 345	II	USA, human blood, 1938	Acute	81EP
		American Q Dyer¶	I	USA, human blood, 1938	Acute	75EP/1GP
		Australia QD RSA425	II	Australia, human blood, ~1939	Acute	177EP
		Turkey RSA333	II	Turkey, human blood, 1948	Acute	31EP
		African RSA334¶	I	Central Africa, human blood, 1949	Acute, Congolese Red Fever	3HP/4EP
		Giroud RSA431¶	I	Central Africa, human blood, 1949	Acute, Congolese Red Fever	2GP/2EP
		El Tayeb RSA342	I	Egypt, tick, 1967	—	4GP/2EP
		Panama RSA335	I	Panama, chiggers, 1961	—	4EP
		California 33 RSA329	I	California, cow's milk, 1947	Persistent	6EP
		California 16 RSA350	II	California, cow's milk, 1947	Persistent	38EP
		Ohio 314 RSA270¶	I	Ohio, cow's milk, 1956	Persistent	4EP
		Ohio 314 RSA338¶	II	Ohio, cow's milk, 1956	Persistent	42EP
II	QpH1	<i>M-44 RSA459</i>	II	Italo-Greek, 'Grita', ~1945	Acute	?/2EP
		M-44 Q141¶	II	Italo-Greek, 'Grita', ~1945	Acute	?/1GP/2EP
		Henzerling RSA331	II	Italy, human blood, 1945	Acute	36EP
III	QpH1	<i>Idaho Goat Q195</i>	I	Idaho, goat, 1981	Abortion	2EP
		Idaho Goat	I	Idaho, goat placenta, 1975	Abortion	5EP
		Koka	I	Ethiopia, tick, 1963	—	1GP/6EP
IV	QpRS	<i>MSU Goat Q177</i>	I	Montana, goat cotyledon, 1980	Abortion	GP/2EP
		Canada Goat Q218	I	Ontario, Canada, goat spleen, 1981	Abortion	1GP/1EP
		Idaho Sheep 80-1	I	Idaho sheep liver, 1980	Abortion	1GP/4EP
		K Q154	I	Oregon, human heart valve, 1976	Endocarditis	HV/2EP
		P Q173	I	California, human heart valve, 1979	Endocarditis	HV/2EP
		F Q228	I	Washington, human heart valve, 1982	Endocarditis	HV/3EP
		H WSU101	I	California, human heart valve, 1986	Endocarditis	HV/2EP
V	NP	<i>L Q216</i>	I	Nova Scotia, human heart valve, 1981	Endocarditis	HV/2EP
		G Q212	I	Nova Scotia, human heart valve, 1981	Endocarditis	HV/2EP
		S Q217	I	Montana, human liver biopsy, 1981	Hepatitis	BX/2EP
		Ko Q229	I	Nova Scotia, human heart valve, 1982	Endocarditis	HV/2EP
VI	QpDG	<i>Dugway 7E22-57</i>	I	Utah, rodents, 1958	—	3EP
		Dugway 7E9-12	I	Utah, rodents, 1958	—	3EP
		Dugway 7E65-68	I	Utah, rodents, 1958	—	3EP

* As defined by restriction enzyme banding patterns described herein.

† Plasmids described (Samuel *et al.*, 1985).

‡ Provided by Rocky Mountain Laboratories, National Institute of Allergy and Infectious Disease, Hamilton, MT, USA. Reference strains are italicized.

§ Determined by complement block titration, M. G. Peacock, Rocky Mountain Laboratories.

|| Preceding number indicates passage number; GP, guinea-pig passage; TC, tissue culture; EP, egg passage; HP, hamster passage; HV, heart valve; BX, liver biopsy; ?, passage prior to receipt in authors' laboratory not known.

¶ Passage history variants.

Phase I antigens easily removed.

when chromosomal DNA was digested with *EcoRI* and probed with radiolabelled plasmid QpH1. Although a minor component of plasmid DNA was resolved with the chromosomal DNA, the majority of differences in the chromosomal DNA fingerprints (Fig. 4b) are not due to the plasmid variability previously reported (Samuel *et al.*, 1985). For example, the banding patterns of plasmids QpH1, QpRS, and QpDG digested with *BamHI* each have only three fragments, which are identical in size, under 3.5 kb (data not shown). Therefore, the differences

seen in the banding patterns of *BamHI* chromosomal digests are not due to plasmid differences. On the other hand, S Q217 and MSU Goat Q177 have several differences in their chromosomal DNA fingerprints, such as a 3.4 kb, a 1.9 kb and a 1.5 kb band found in S Q217 (Fig. 4b, lane B) but not found in MSU Goat Q177. S Q217 is grouped with those isolates which do not contain a plasmid, although it does have plasmid-related sequences integrated into the chromosome (E. Savinelli, unpublished). The correlation between the genomic

Fig. 4. (a) Autoradiograph of plasmid QpH1 DNA probe hybridization with DNA of one *C. burnetii* isolate from each genomic group. Chromosomal DNA extracted from each organism was digested with *EcoRI* and separated by electrophoresis on a 0.9% agarose gel in Tris/acetate buffer. A Southern blot was prepared from the agarose gel and hybridized with a nick-translated QpH1 probe using conditions allowing for approximately 20% mismatch of sequences. Lanes: (A) Dugway 7E9-12; (B) S Q217; (C) MSU Goat Q177; (D) Nine Mile RSA493 phase I; (E) M-44 RSA459; (F) Idaho Goat Q195. Markers indicate fragment size of λ phage DNA digested with *HindIII*. (b) *Bam*HI restriction endonuclease digestion of the DNA of one *C. burnetii* isolate from each genomic group. Chromosomal DNA was digested with *Bam*HI and fragments separated by SDS-PAGE as described in Fig. 1. Lanes as in (a). Markers indicate fragment sizes of λ phage DNA digested with *HindIII* and of ϕ X174 phage DNA digested with *Hae*III.

grouping shown in Fig. 4(b) and the plasmid type (Fig. 4a) confirms the uniqueness of each isolate group but does not quantify their relatedness.

Quantification of group variation measured by DNA-DNA hybridization

To quantify the relatedness of isolates within and between chromosomal groups, DNA-DNA hybridiza-

tion analysis on selected isolates was performed (Table 2). This type of analysis has previously been performed with various *Rickettsia* spp. and was used to assign relatedness at the strain level (Myers & Wisseman, 1980). Two isolates, Nine Mile RSA493 and Dyer RSA345, were included in the present comparison to demonstrate relatedness within a genomic group. In fact, Nine Mile RSA493 and Dyer RSA345 are essentially passage history variants of the same original isolate. Group VI

Table 2. DNA-DNA hybridization between isolate groups of *C. burnetii*

The degree of hybridization was determined twice for each pair of strains, each time with separately prepared DNA. The *D* value is a mean of these two measurements expressed $\pm$ the standard deviation of the mean. ND, Not determined.

Reference DNA	<i>D</i> value (%)			
	Nine Mile*	Dyer†	Dugway‡	MSU Goat§
Nine Mile*	—	101 $\pm$ 1	90 $\pm$ 5	92 $\pm$ 4
MSU Goat§	92 $\pm$ 4	ND	95 $\pm$ 2	—

* RSA493, genomic group I.

† RSA345, genomic group I.

‡ 7E22-57, genomic group VI.

§ Q177, genomic group IV.

(Dugway 7E22-57) and group IV (MSU Goat Q177) isolates were included to measure relatedness between distinct genomic groups on the basis of DNA fragment analysis. Due to the large amount of DNA required for hybridization analysis, isolates from groups II, III and V were not included in this study, as they were typically more difficult to propagate to high titre. Within one group, Nine Mile RSA493 and Dyer RSA345 had 101 $\pm$ 2% homology as measured by the hybridization value (*D*). This result would be predicted from their aetiological relationship. Between genomic groups, *D* values ranged from 90 to 95%. These values were an average of two independent experiments for each comparison, with separately prepared DNA used for each experiment. The standard deviation of the average hybridization (*D*) between the two experiments ranged from 2% to 5% but, because of the small number of samples in the comparison of experiments ($n = 2$), these values were not statistically significantly different ($P < 0.1$) between groups.

Discussion

C. burnetii organisms cause both acute and chronic disease in humans and have been isolated from a wide variety of warm-blooded animals and from arthropods. Previous studies have shown plasmid (Samuel *et al.*, 1985; Vodkin *et al.*, 1986) and LPS (Hackstadt, 1986) differences among isolates, which have allowed the isolates to be grouped. This grouping of isolates appears to correlate with their pathogenic potential, but the degree of variability between groups has not been determined. The present study illustrates that restriction enzyme digestion of chromosomal DNA (Fig. 4*b*) can also be used to differentiate these isolates and that the

grouping based on plasmid type (Fig. 4*a*) is retained. Standard agarose gel separation of RE-generated DNA fragments is useful in that differences are revealed (O'Rourke *et al.*, 1985), but this method is limited because of inadequate band resolution. The use of SDS-PAGE analysis and silver staining as described by McClenaghan *et al.* (1984) allows better fragment pattern resolution, and the number of *C. burnetii* groups is increased to six. Because the technique is highly sensitive, isolates within a group and isolates from different groups are easily distinguishable (Fig. 1).

A reproducible difference between egg-grown phase I and phase II variants of the Nine Mile isolate was previously demonstrated by agarose gel electrophoresis of *Hae*III-digested DNA, namely the presence of a DNA fragment of approx. 4.6 kb in digests of DNA from phase I organisms which was not found in the DNA fingerprint of phase II organisms; however, no other differences were detected when a variety of other restriction enzymes were used to digest DNA from these variants or from phase variants of other strains (O'Rourke *et al.*, 1985). This would seem to suggest that the level of change seen with RE polymorphisms is infrequent during repeated egg or tissue culture passage. This suggestion is further supported by the fact that no differences in DNA fragment fingerprints were observed between passage-history variants of six *C. burnetii* isolates when analysed by the more sensitive SDS-PAGE procedure used in this study (Fig. 2). Therefore, the variability seen between the groups identified here probably represents significant evolutionary divergence.

The DNA-DNA hybridization data presented here also suggested separation of the isolate groups. While a comparison of the hybridization values for the two experiments did not indicate that the values were statistically significant ($P < 0.1$), these values clearly suggest real biological differences. Although DNA-DNA hybridization has been criticized as a technique for determining the relatedness of different groups, this procedure has previously been used with other rickettsial species to differentiate between species and also between strains within a species (Myers & Wisseman, 1980). That study found differences in hybridization (*D*) from 0 to 8% for rickettsiae of the same species that were previously separated into distinct strains by serological criteria. Thus the three genomic groups tested in our study, viz. groups I, IV and VI, represent three distinct *C. burnetii* strains. While the results suggest that group V is also a separate strain, the data are not clear on whether groups I, II and III comprise one or three strains. The isolates in these three groups have the same plasmid type, but have different genomic DNA fingerprints and LPS (Hackstadt, 1986) banding patterns. Methods such as filter hybridization (Crosa *et al.*, 1973), restriction

fragment length polymorphism using randomly generated probes (Nei, 1987) and multilocus enzyme analysis (Selander *et al.*, 1986) could be used to determine the genetic distance between these strains more precisely.

Finally, we propose to use these data to organize individual *C. burnetii* stocks according to a specific nomenclature system. Individual stocks are currently maintained and separately identified that differ with respect to source of original isolation, passage history, and LPS type (phase I or phase II). We propose to use the word 'strain' to indicate the six separate groups as defined previously by plasmid and LPS type, and, in this report, by the banding patterns of RE-digested chromosomal DNA.

This work was supported by Public Health Service grant R01 A120190 from the National Institutes of Health.

References

- BACA, O. G. & PARETSKY, D. (1983). Q fever and *Coxiella burnetii*: a model for host-parasite interactions. *Microbiological Reviews* **47**, 127-149.
- CROSA, J. H., BRENNER, D. J. & FALKOW, S. (1973). Use of a single-stranded specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *Journal of Bacteriology* **115**, 904-911.
- HACKSTADT, T. (1986). Antigenic variation in the phase I lipopolysaccharide of *Coxiella burnetii* isolates. *Infection and Immunity* **52**, 337-340.
- HENDRIX, L. & MALLAVIA, L. P. (1984). Active transport of proline by *Coxiella burnetii*. *Journal of General Microbiology* **130**, 2857-2863.
- HERRING, A. J., INGLIS, N. F., OJEH, C. K., SNODGRASS, D. R. & MENZIES, J. D. (1982). Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. *Journal of Clinical Microbiology* **16**, 473-477.
- LAEMMLI, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680-685.
- DELAY, J. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *European Journal of Biochemistry* **12**, 133-142.
- MALLAVIA, L. P., FRAZIER, M. E., RICHARDSON-CARLSON, R. & SAMUEL, J. E. (1985). Grouping of *Coxiella burnetii* isolates by restriction endonuclease analysis. In *Abstracts of the Annual Meeting of the American Society of Microbiology*, D79, p. 67.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- MCCLENAGHAN, M., HERRING, A. J. & AITKEN, I. D. (1984). Comparison of *Chlamydia psittaci* isolates by DNA restriction endonuclease analysis. *Infection and Immunity* **45**, 384-389.
- MYERS, W. F. & WISSEMAN, C. L., JR (1980). Genomic relationship among the Typhus group of Rickettsiae. *International Journal of Systematic Bacteriology* **30**, 143-150.
- NEI, M. (1987). *Molecular Evolutionary Genetics*. New York: Columbia University Press.
- O'ROURKE, A. T., PEACOCK, M., SAMUEL, J. E., FRAZIER, M. E., NATVIG, D. O., MALLAVIA, L. P. & BACA, O. (1985). Genomic analysis of phase I and II *Coxiella burnetii* with restriction endonucleases. *Journal of General Microbiology* **131**, 1543-1546.
- PEACOCK, M. G., PHILIP, R. N., WILLIAMS, J. C. & FAULKNER, R. S. (1983). Serological evaluation of Q fever in humans: enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. *Infection and Immunity* **41**, 1089-1098.
- SAMUEL, J. E., FRAZIER, M. E., KAHN, M. L., THOMASHOW, L. S. & MALLAVIA, L. P. (1983). Isolation and characterization of a plasmid from phase I *Coxiella burnetii*. *Infection and Immunity* **41**, 488-493.
- SAMUEL, J. E., FRAZIER, M. E. & MALLAVIA, L. P. (1985). Correlation of plasmid type and disease caused by *Coxiella burnetii*. *Infection and Immunity* **49**, 775-779.
- SELANDER, R. K., CAUGANT, D. A., OCHMAN, H., MUSSER, J. M., GILMOUR, M. N. & WHITTAM, T. S. (1986). Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. *Applied and Environmental Microbiology* **51**, 873-884.
- TOPPING, N. H., SHEPARD, C. C. & HUEBNER, R. J. (1946). Q fever. An immunological comparison of strains. *American Journal of Hygiene* **44**, 173-182.
- VODKIN, M. H., WILLIAMS, J. C. & STEPHENSON, E. H. (1986). Genetic heterogeneity among isolates of *Coxiella burnetii*. *Journal of General Microbiology* **132**, 455-463.