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Evaluation of randomly amplified polymorphic DNA (RAPD) for discrimination of *Coxiella burnetii* ruminant strains isolated in France

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Coxiella burnetii is the causative agent of Q fever, a worldwide zoonosis. Ruminant species represent the main reservoir of the bacterium, as high levels of the bacteria are shed in birth products and other excreta. Human infection generally occurs after inhalation of contaminated aerosols [1]. The availability of methods to study the distribution and the spread of a given *C. burnetii* strain, from different geographical areas or hosts, would promote a better understanding of the epidemiology of this pathogen. Several genetic typing methods for *C. burnetii* have been evaluated and appeared to be useful tools for epidemiological and phylogenetic purposes. However, RAPD [2,3] has never been evaluated for *C. burnetii* typing. In this work, a RAPD protocol was used to evaluate the genetic diversity among *C. burnetii* ruminant strains in comparison to published MLVA data [4].

Ten French *C. burnetii* isolates obtained from goats, sheep, cows and the reference strain Nine Mile, were used for RAPD typing (Table 1). Whole DNA was extracted using the Qiagen Dneasy kit (Courtaboeuf, France), following the manufacturer's protocol. To assess optimal RAPD conditions several parameters were tested, for instance using a range of $MgCl_2$ concentrations (from 1.5 to 5.5 mM), or different brands of *Taq* polymerase. RAPD PCRs were performed using a set of four 10-mer primers (P4M, 5′-AAGACGCCGT-3′; PR5, 5′-AGTCGTCCCC-3′; PR10A, 5′- AGGGCCGTCT-3′; and PR12A, 5′-CAGCTCACGA-3′). Unless specified, the optimised PCR conditions were, for a final volume of 25 μL in dH_2O: 1X *Taq* polymerase buffer (without $MgCl_2$); 3 mM $MgCl_2$; 0.2 mM each dNTPs; 1U of *Taq* polymerase; 0.5 μM 10-mer primer; and 10 ng of DNA (all the reagents were purchased from Invitrogen, Cergy Pontoise, France). Negative controls consisting of dH_2O only were included in each run. Amplifications were performed in an Eppendorf Mastercycler (Le Pecq, France), programmed for an initial denaturation step of 5 min at 94°C, 45 cycles of 96°C for 30 s, 37°C for 30 s, 72°C for 90 s and a final extension step at 72°C for 5 min. The PCR products were separated on 0.8% agarose gel containing ethidium bromide, visualised and photographed under UV light. Gel analysis was performed using the Quantity One 1-D Analysis software from Bio-Rad (Marnes la Coquette, France). An RAPD type was defined after the combination of the patterns obtained with the four primers used in this study (Table 1). The reproducibility of the method was evaluated by repeating the same assay several times, by different manipulators, as well as by using independent DNA preparations.

Briefly, RAPD profiles were stable when varying $MgCl_2$ concentration. However, a higher yield of amplification, estimated by the intensity of the bands observed on the gels, was observed using 2.5–3.5 mM $MgCl_2$. This yield decreased when using higher concentrations of $MgCl_2$ (data not shown). A $MgCl_2$ concentration of 3 mM was therefore chosen for subsequent analyses. Different RAPD patterns were observed when different brands of *Taq* polymerases were used (data not shown). The Hot start platinum *Taq* polymerase (Invitrogen) produced the best patterns in our hands and was chosen for all the experiments.

After optimisation of our RAPD protocol, the use of each primer generated distinct polymorphisms allowing differentiation of the studied strains. Strains from neighbouring flocks, that were indistinguishable using MLVA analysis, could be discriminated using RAPD typing (Table 1). For instance, CbB2 and CbB5 are two strains obtained in 2001 from neighbouring flocks, but were isolated from cows displaying different clinical signs (Table 1). CbB2 was isolated from a case of metritis and CbB5 from an aborted cow.

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Table 1. List, characteristics and fingerprinting patterns of *Coxiella burnetii* strains studied with MLVA and RAPD typing

Strain	Host	Tissue source	Clinical signs	Country (area)	MLVA Type	RAPD patterns				Combined RAPD type
						P4M	PR5	PR10A	PR12A	
Nine Mile	Tick	None	None	USA (Montana)	4	A	A	A	A	1
CbB1	Cattle	Placenta	Abortion	France (61)	2	B	E	C	C	2
CbB4	Cattle	Placenta	Abortion	France (61)	2	B	G	F	E	3
CbB7	Cattle	Placenta	Abortion	France (61)	2	B	G	F	F	4
CbB5	Cattle	Milk	Abortion	France (76)	2	B	G	F	F	4
CbB2	Cattle	Milk	Metritis	France (76)	2	B	F	E	D	5
CbC1	Goat	Placenta	Abortion	France (03)	2	B	B	B	B	6
CbC2	Goat	Milk	None	France (79)	7	A	C	C	C	7
CbC5	Goat	Milk	Abortion	France (82)	7	C	D	D	D	8
CbO1	Sheep	Placenta	Abortion	France (37)	19	D	H	E	G	9
CbO184	Sheep	Placenta	Abortion	France (06)	19	E	I	G	C	10

These strains exhibited similar MLVA patterns but could be distinguished by RAPD. Similarly, the two strains from ovine CbO1 and CbO184, isolated from different areas in France and at different times, were different whatever RAPD primer used but were similar by MLVA typing. Moreover, the strains isolated from goats appeared to be polymorphic, and differed from strains obtained from cattle and sheep.

This preliminary study, using a standardised protocol, showed that the RAPD method was reproducible and suitable for generating polymorphisms among closely related *C. burnetii* strains. Several molecular typing methods have been used successfully for strain-typing of *C. burnetii*. Recently, highly discriminating methods such as MLVA [4] and MST [5] typing allowed identification of up to 36 distinct *C. burnetii* genotypes. The strains used for this study have been already typed by MLVA [4] and by MST in our laboratory. Similar results were obtained: the 10 strains were divided into four distinct groups (data not shown) whereas further discrimination among these strains was achieved using our RAPD protocol. To conclude, RAPD seems a promising tool for epidemiological studies of Q fever. It needs to be further assessed both in human and ruminant outbreaks as a complementary investigation technique to other genotyping methods, in order to facilitate the study of the spread of a given isolate within and between flocks.

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