Differentiation of *Chlamydia psittaci* and *C. pecorum* Strains by Species-Specific PCR

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Sequence analyses of the 5′ ends of the 60-kDa cysteine-rich outer membrane protein genes (*Omp2*) of *Chlamydia psittaci* and *Chlamydia pecorum* strains indicate that these species have approximately 70% nucleotide identity. On the basis of this sequence information, PCR primers were designed to allow the specific amplification of DNA extracted from *C. psittaci* S26/3 (abortion strain), P94/1 (pigeon strain), and *C. pecorum* W73 (fecal strain) in one reaction tube. By using nested reactions (with primers PCR-D1 and PCR-D2 followed by the specific primers and PCR-D2), 0.6, 0.2, and 8 inclusion-forming units of S26/3, P94/1 (both diluted in tissue culture-negative placental material), and W73 (diluted in culture-negative fecal material) per ml, respectively, were detected. The differentiation of *C. psittaci* and *C. pecorum* strains of ovine and bovine origins was carried out, and the results were in agreement with those obtained from *Alu*I restriction enzyme analysis of DNA amplified from corresponding strains by PCR. This approach allows the simultaneous detection and typing of *C. psittaci* and *C. pecorum* strains and the identification of samples containing both species.

The genus *Chlamydia* comprises four species, *C. psittaci*, *C. trachomatis* (26), *C. pneumoniae* (13), and *C. pecorum* (12). The most important diseases caused by chlamydiae in humans are trachoma and urogenital infections (*C. trachomatis*), respiratory infections due to *C. pneumoniae*, and psittacosis caused by *C. psittaci* (29). In animals, *C. psittaci* and *C. pecorum* give rise to a wide variety of conditions, including abortion, pneumonia, enteritis, polyarthritis, encephalomyelitis, and conjunctivitis (32).

Methods of interspecies differentiation based on inclusion morphology, growth characteristics (6, 25, 31), and serology using monoclonal antibodies (1, 4, 11, 13, 33) are well documented. Interspecies DNA homologies were found to range from approximately 5 to 20%, as determined by restriction enzyme (RE) analysis of whole genomic DNA (6, 12, 13). Previous studies have demonstrated that PCR followed by RE analysis of amplified DNA can be used to detect and differentiate chlamydial species (5, 8, 10, 16, 27). Sequence analysis and RE profiles of DNAs amplified from the major outer membrane protein (MOMP) gene of *C. psittaci* strains indicate that ruminant abortion, guinea pig inclusion conjunctivitis (GPIC), and feline isolates possess relatively homogeneous MOMP sequences compared with *C. pecorum* isolates, which exhibit considerable sequence heterogeneity in this gene.

Kaltenboeck et al. (19) devised a multiplex PCR in which MOMP genes were initially amplified with genus-specific primers and then were subjected to secondary amplification with primers which gave rise to different-size products representing different MOMP genotype groupings. PCR amplification of the 60-kDa cysteine-rich outer membrane protein gene *Omp2* followed by RE analysis of amplified products allowed the differentiation of three chlamydial species and strains within those species (2, 36). Analysis of these published data indicate less sequence variability in *Omp2* than in the MOMP gene, with *C. psittaci* ovine abortion strains, *C. psittaci* avian strains, and *C. pecorum* strains forming separate groupings. Minor differences in the migration patterns of DNA fragments,

produced by RE digestion of PCR products amplified from the 16S rRNA gene, allowed *C. psittaci* (abortion) strains to be distinguished from *C. pecorum* strains (2, 15).

Omp2 nucleotide sequences of *C. psittaci* S26/3 (ovine abortion strain) and *C. pecorum* W73 (ovine enteric strain) were determined in a previous study (30). In addition, partial *Omp2* nucleotide sequences of *C. psittaci* P94/1 (pigeon strain), *C. pecorum* P787 (arthritis strain), and E58 (encephalomyelitis strain) were determined. Analyses of these data, together with other published data (9, 34, 35), indicate the presence of an interspecies variable region flanked by short highly conserved regions at the 5′ ends of this gene. This sequence information was used in the present study to design species- and intraspecies-specific primers to facilitate the simultaneous detection and typing of chlamydial isolates.

TABLE 1. Details of *C. psittaci* and *C. pecorum* strains

Isolate	Host	Clinical condition of host yielding isolate
S26/3[a]	Sheep	Abortion
C94/1	Sheep	Abortion
P94/1	Pigeon	Ornithosis
VF88/2122	Budgerigar	Psittacosis
GPIC[a]	Guinea pig	Conjunctivitis
LV350/93[b]	Bovine	Abortion
ZC53[c]	Sheep	Normal (feces)[e]
W73	Sheep	Normal (feces)[e]
C95/38	Sheep	Normal (feces)[e]
P787[a]	Sheep	Arthritis
E58[d]	Bovine	Encephalomyelitis
PV3056/3[f]	Bovine	Metritis

[a] From G. Jones, Moredun Research Institute.
[b] From B. Biolatti, Department of Animal Pathology, Turin, Italy.
[c] From H. Phillips and M. Clarkson, Department of Veterinary Clinical Science and Animal Husbandry, University of Liverpool, Liverpool, England.
[d] From the American Type Culture Collection (strain VR628).
[e] From fecal samples.
[f] From S. Magnino, P. G. Vigo, and M. Fabbi, Instituto Zooprofilattico Sperimentale della Lombardia e Pavia, Italy.

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TABLE 2. *Omp2* PCR primers

Primer	Sequence
Nested primers	
PCR-D1...........1	5′ CAA ACT CAT CAG ACG AG 3′ 17
PCR-D2...........582	5′ CCT TCT TTA AGA GGT TTT ACC 3′ 562
Selective primers[a]	
PCR-Ab...........253	5′ TCA GTG CCA ATC CGT CGA TA 3′ 272
PCR-Av...........192	5′ GAC AAG AAC AAA AAC ATA CTG AA 3′ 214
PCR-Pe............109	5′ TAT TGC AAA TGC AGA ATC TAA GC 3′ 131

[a] PCR-Ab (abortion), PCR-Av (avian), and PCR-Pe (pecorum) are sense primers.

MATERIALS AND METHODS

Chlamydial strains. Details of the chlamydial strains analyzed in this study are shown in Table 1. All strains were propagated in developing chick embryos and yolk sac material was harvested as previously described (24). Strains were titrated in McCoy cells treated with cycloheximide (1 μg/ml), and the titers were expressed as inclusion-forming units (IFU) per milliliter.

DNA extraction. DNA was extracted from yolk sac material infected with the chlamydia isolates described in Table 1 as described by Anderson et al. (2). Uninfected yolk sac material was used as a negative control throughout this study. Briefly, 100 μl of yolk sac was mixed with 400 μl of lysis buffer (50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1% sodium dodecyl sulfate and 200 μg of proteinase K per ml) and incubated either at 37°C overnight or at 55°C for 2 h. The DNA samples were extracted twice with phenol-chloroform-isoamyl alcohol, ethanol precipitated, and resuspended in distilled water. DNA used in sensitivity experiments was extracted from serial dilutions (10-fold) of *C. psittaci* S26/3 (6×10^7 IFU/ml)- and P94/1 (2×10^6 IFU/ml)-infected yolk sac prepared in PCR and tissue culture-negative ovine placental material. DNA was extracted from each dilution as described above. Serial dilutions of W73 (8×10^6 IFU/ml)-infected yolk sac were prepared in tissue culture-negative ovine feces. DNA was extracted from each dilution with a commercial DNA extraction kit, Nucleiclean (Sigma), according to the manufacturer's instructions. Tissue culture-negative placental or fecal material used in dilutions was prepared by homogenizing approximately 1 g of either material in 5 to 10 ml of phosphate-buffered saline.

PCR protocol. The sensitivity of PCRs containing the specific primers (sense) together with PCR-D2 (antisense) (Table 2) was determined either in primary reactions or in nested reactions. Generally, 2-μl aliquots of DNA extracted from serial dilutions of isolates S26/3, P94/1, and W73 were used in PCRs in final reaction volumes of 50 μl containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X, 2 mM MgCl₂, 200 μM (each) deoxynucleoside triphosphates, and 0.5 U of *Taq* polymerase (Promega). In primary reactions, DNA was amplified by using the specific primers PCR-Ab, PCR-Av, or PCR-Pe and PCR-D2 (0.2 μM) (Table 2). In nested PCRs, DNA was initially amplified by using PCR-D1 and PCR-D2 (0.5 μM). An aliquot of this product was subsequently reamplified in a

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            PCR-D1
A22/M     1 CAAACTCATCAGACGAGTAGTTACGGTCCTCGCGCCTAACTAGTATGGCGAGTTCATTCGC
6BC       1 ............................................................
P94/1     1 ............................................................
S26/3     1 ............................................................
C94/1     1 ............................................................
IOL-207   1 ..................................T........G............GC....
W73       1 .........T..T..............................A........C....
P787      1 .........T..T..............................A........C....
E58       1 .........T..T..............................A........C....

A22/M    61 CAGCGGGAAGATAGAGGCCGCTGCTGCAGAGTCTCTTGCTACAAGATTCATTGCCAGTAC
6BC      61 ............................................................
P94/1    61 ............................................................
S26/3    61 ............................................................
C94/1    61 ............................................................
IOL-207  61 .......GGT..............TA..........GAT...T.AGA..G.C..T...G.
                                                          PCR-Pe
W73      61 .......GGA..............TA..........GATG..T.A.A.T....A.A.G.
P787     61 .......GGA..............TA..........GATG..T.A.A.T....A.A.C.
E58      61 .......GGA..............TA......C...GATG..T.A.A.T.....A.A.G.

A22/M   121 TGAAAACGCAGATGACAATGTTTTTCAAGCAACAGCCAAGAAAGTTAGATTTGGCCGTAA
6BC     121 .......T....................................................
P94/1   121 ............................................................
S26/3   121 C......T..A...............G.................................
C94/1   121 C......T..A...............G.................................
IOL-207 121 _.G....AA...CC.GC.CC.G..C.T._TG.....G......G........C...TC...G.
W73     121 A...TCTAAGCG..CAGCA.A.CA.A..AA.GA.......C.TAA.C.TAACAAAAAC.
P787    121 A...TCTAAGCG..CAGCA.A.CA.A..AA.GA.......C.TAA.C.TAACAAAAAC.
E58     121 A...TCTAAGCG..CACCA.A.CA.A..AA.GA.......C.TAA.C.TAACAAAAAC.

              PCR-Av        AluI
A22/M   181 CAAAAATCAAAGACAAAGAACAAAAACATACTGAAGCTTTCTGTGATAAAGAATTTTATCC
6BC     181 ..................................G.....................
P94/1   181 ............................................................
S26/3   181 .......................................GC.................
C94/1   181 .......................................GC.................
IOL-207 178 A..T..A...CC.GTT..........AGCCG..GT....T.............
W73     181 TTC...AGCT.C.A..C.CA.C.GCAGAT..A.CC...CA...TG.G.TA..GAG.TC..
P787    181 TTC...AGCT.C.A..C.CA.C.GCAGAT..A.CC...CA...TG.G.TA..GAG.TC..
E58     181 TTC...AGCT.C.A..C.CA.C.GCAGAT..A.CC...CA...TG.G.TA..GAG.TC..
            AluI

A22/M   241 TTGTGAAGGTGGCCAGTGCCAACCAGTAGACGCTACACAAGAATCTTGCTACGGCAA
6BC     241 ............................................................
P94/1   241 ............................................................
                                PCR-Ab
S26/3   241 ...C........T.......T.C..C..TA...........................
C94/1   241 ...C........T.......T.C..C..TA...........................
IOL-207 238 C.......AG..A.GA..T.....T.....G..CAG.....G...........A.G
W73     241 ......AGAAGA.T......A......GAGCCG.............T...T..
P787    241 ......AGAAGA.T......A......GAGCCG...........T...T..
E58     241 ......AGAAGA.T......A......GAGCCG...........T...T..
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A22/M   298 AATGTATCGTGTCCGTGTTAACGATGACTGTAACGTTGAAATCAGCCAATCTGTACCTGA
6BC     298 ............................................................
P94/1   298 ............................................................
                                                  AluI
S26/3   298 ........................................G....T......G.......
C94/1   298 ........................................G....T......G.......
IOL-207 295 .T......C...AAAA..A.......T..C....A......TT...G..C..T..A.
W73     298 G........CCA.AAAA..G..T..C..T..C..T..A...C.A..T......T....
P787    298 G........CCA.AAAA..G..T..C..T..C..T..A...C.A..T......T....
E58     298 G........CCA.AAAA..G..T..C..T..C..T..A...C.A..T......T....

A22/M   358 ATATGCAACAGTAGGATCTCCTTATCCTATTGAAATTCTCGCTGTAGGTAAAAAACGATTG
6BC     353 ............................................................
P94/1   358 ............................................................
S26/3   359 ...................................T....................
C94/1   358 ...................................T....................
IOL-207 355 ...C..T..T.............C.........C..T..A....C..........
W73     358 ...........C.........A.........T.A..GA.I..A........C.
P787    358 ...........C.........A.........T.A..GA.T..A........C.
E58     358 ...........C.........A.........T.A..GA.T..A........C.

                      AluI
A22/M   418 CGTTAATGTTGTGATTACTCAACAGCTTCCTTGCGAAGTTGAGTTTGTCAGCAGTGATCC
6BC     418 ............................................................
P94/1   418 ..........................****..............................
S26/3   418 ...............C..........A.................................
C94/1   418 ...............C..........A.................................
IOL-207 415 T...G..........A........A........C...A..C..A........
W73     418 ...A........C..C..A........T....CG..A....T.....C...
P787    418 ...A........C..C..A........T....CG..A....T.....C...
E58     418 ...A........C..C..A........T....CG..A....T.....C...

A22/M   478 TGCGACAACACCAACCTCGGATAGCAAATTAATCTGGACAATTGATCGCTTAGGTCAAGG
6BC     478 ............................................................
P94/1   478 ............................................................
S26/3   478 ...............A...................T...................
C94/1   478 ...............A...................T...................
IOL-207 475 A.AA......T..T..AAGT...G.G.......G.......A...C......C.G...GC...
W73     478 ..AA..T......G.A...G.G.....G..G.....A...C......T....A...
P787    478 ..AA..T......G.A...G.G.....G..G.....A...C......T....A...
E58     478 ..AA..T......G.A...G.G.....G..G.....A...C......T....A...

                                        PCR-D2
A22/M   538 TGAAAAATGCAAAATTACCGTTTGGGTAAAACCTCTTAAAGAAGG
6BC     538 .........................................
P94/1   538 .........................................
S26/3   538 .........................................
C94/1   538 .........................................
IOL-207 535 A..T............T..A...............
W73     538 C....G..........T...
P787    538 C....G..........T...
E58     538 C....G..........T...
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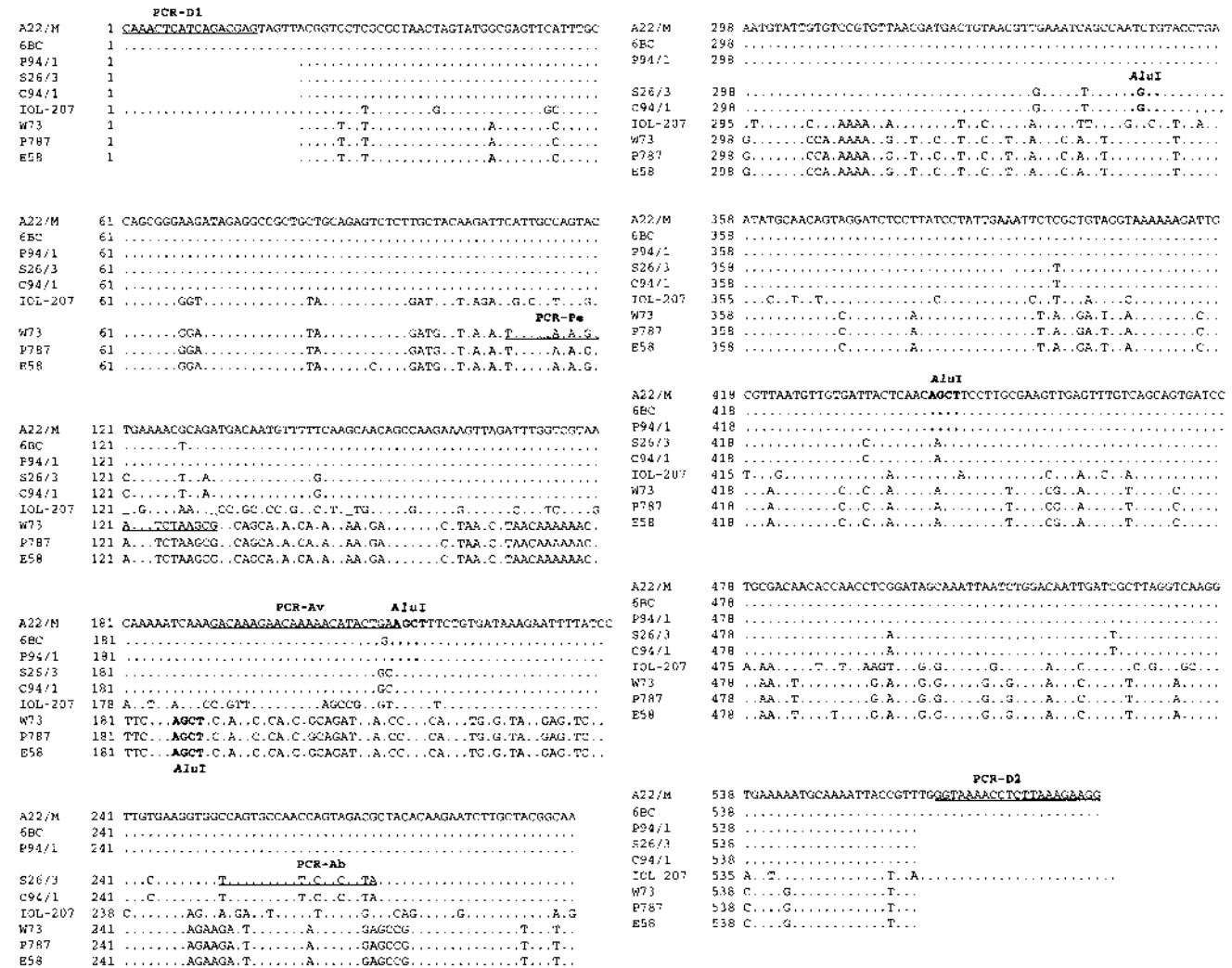
FIG. 1. Alignment of partial *Omp2* nucleotide sequences derived from *C. psittaci* and *C. pecorum* strains compared with those from *C. psittaci* A22/M (35) and 6BC (9) and *C. pneumoniae* IOL-207 (34). The locations of *Alu*I RE sites are indicated in boldface type. Also indicated are the positions of the specific primers PCR-Ab (abortion), PCR-Av (avian), and PCR-Pe (pecorum).

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A22/M    1 KLTRRVVTVLALTSKASSFASGKIEAAAAESLATRSIASTENADDNVEQATAKKVRFGRN
6BC      1 ............................................S...............
P94/1    1 ..........................................................
S26/3    1 .................................SN...L....................
C94/1    1 .................................SN...L....................
IOL-207  1 ...........C...G.....V....I.KIV..A.TKPAP..PM....LV.R
P787     1 .........T.....G....V....M.KI..NA.SKRAADHKKE..HN.NKKH
W73      1 .........T.....G....V....M.KI..NA.SKRAADHKKE..HN.NKKH
E58      1 .........T.....G....V.P.M.KI..NA.SKRAADHKKE..HN.NKKH


A22/M   61 KNQRQEQKHTEAFCDKEFYPCEGGQCQPVDATQESCYGKMYCVRVNDDCNVRISQSVPS
6BC     61 ..........G...............................................
P94/1   61 ..........................................................
S26/3   61 ..........G...............S..T.....................A.......
C94/1   61 ..........G...............S..T.....................A.......
IOL-20  60 NK.PV...SRG............E.P....E.Q......RL.S.K.........C.....
P787    61 SKATKHNSRSNPS.CDKEF...EKN..Q.ESR....F....SIK........L......
W73     61 SKATKHNSRSNPS.CDKEF...EKN..Q.ESR....F....SIK........L......
E58     61 SKATKHNSRSNPS.CDKEF...EKN..Q.ESR....F....SIK........L......


A22/M  120 YATVGSPYPIEILAVGKKDCVNVVITQQLPCEVEFV5SDPATTPTSDSKLLWTIDRLGQG
6BC    120 ..........................................................
P94/1  120 ..........................................................
S26/3  120 ...................................................C.......
C94/1  120 ...................................................C.......
IOL-207 119 .............I.......D.........A.......E.......G..V.K......A.
P787   120 .............I................A.......E...A.G..M.K.........
W73    120 .............I................A.......R...A.G..M.K.........
E58    120 .............I................A.......E..L.A.G..M.K........


A22/M  180 EKCKITVWVKPLKEG
6BC    180 ...............
P94/1  180 ......
S26/3  180 ......
C94/1  180 ......
IOL-207 179 Q.............
P787   180 .R....
W73    180 .R....
E58    180 .R....
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FIG. 2. Deduced Omp2 amino acid sequences of *C. psittaci* and *C. pecorum* strains.

secondary reaction mixture containing the specific primers and PCR-D2 (0.2 μM). The thermal cycler program used for PCR was 1 min 30 s at 94°C, 1 min at 48°C, and 2 min at 72°C for 25 cycles (nested reactions) followed by 1 min 30 s at 94°C, 1 min at 58°C, and 1 min 30 s at 72°C for 30 cycles (nested and primary reactions). The sensitivity of primary amplifications of DNA using the specific primers was compared with that obtained by using nested primer reactions.

RE analysis. Purified PCR products were digested with *Alu*I RE according to the instructions of the manufacturer (Promega). DNA fragments were separated on 10% polyacrylamide gels and silver stained as described by Anderson et al. (2).

RESULTS

Analysis of partial *Omp2* DNA sequences. The nucleotide and amino acid sequences of the 5′ termini of *Omp2* of *C. psittaci* S26/3, C94/1, and P94/1 and *C. pecorum* W73, P787, and E58 were aligned and are presented in Fig. 1 and 2. *C. psittaci* abortion strains S26/3 and C94/1 had identical nucleotide sequences and greater-than-95% similarity to the avian strains 6BC (9), A22/M (35), and P94/1. The nucleotide sequences of the last two strains were identical. *C. pneumoniae* IOL-207 (36) showed greater-than-77% similarity to *C. psittaci* strains. *C. pecorum* W73 and P787 were identical and differed from E58 by only 2 nucleotides. In general, *C. pecorum* strains showed approximately 72% nucleotide identity to *C. psittaci* strains. At the amino acid level, higher levels of dissimilarities were observed, suggesting that many of the nucleotide changes gave rise to amino acid changes. *C. pecorum* strains displayed approximately 65% identity to *C. psittaci* strains. In general, the amino acid sequences of *C. psittaci* and *C. pecorum* strains were identical over the first 17 amino acids. Considerable interspecies variation occurred between amino acids 18 and 105, and this was followed by a less-variable region spanning amino acids 106 to 185. Everett and Hatch (9) also reported that the Omp2 N-terminal region (first 24 amino acids) of *C. psittaci*

6BC, *C. pneumoniae* IOL-207, and *C. trachomatis* LGV was highly conserved and was followed by a highly variable region spanning amino acids 25 to 106.

RE analysis of *Omp2* PCR products. PCR products amplified by using PCR-D1 and PCR-D2 (36) were digested with *Alu*I. DNA fragments were resolved on 10% polyacrylamide gels (Fig. 3). *C. psittaci* abortion strains yielded fragments of approximately 335 and 240 bp, which were clearly distinct from the RE profiles of *C. pecorum* strains which yielded fragments of approximately 400 and 180 bp. *C. psittaci* P94/1 yielded three fragments of approximately 230, 215, and 140 bp. *C. psittaci* GPIC gave three predominant bands of approximately 350, 140, and 100 bp. Incomplete RE digestion of DNA was evident in some samples. The profiles obtained from all strains were in agreement with their respective nucleotide sequences and with the results obtained by Anderson et al. (2).

Discriminative PCR amplification of *C. psittaci* and *C. pecorum* strains. PCR primers were designed to allow the specific amplification of *C. psittaci* abortion, *C. psittaci* avian, and *C. pecorum* strains on the basis of the nucleotide sequences shown in Table 2. The sensitivities of single or nested PCRs were determined with DNA extracted from serial dilutions of *C. psittaci* S26/3 and P94/1 (6×10^7 and 2×10^6 IFU/ml, respectively) and *C. pecorum* W73 (8×10^6 IFU/ml).

In a single reaction, 6×10^1, 2×10^3, and 8×10^4 IFU of S26/3, P94/1, and W73 per ml, respectively, were detected (Fig. 4A). Nested reactions yielded visible DNA fragments down to 0.6, 0.2, and 8 IFU/ml, respectively (Fig. 4B). The specificity of multiplex PCRs containing the three specific primers and PCR-D2 was assessed with samples containing mixtures of DNA extracted from strains S26/3, P94/1, and W73 (Fig. 5A). Three DNA bands were observed in PCRs containing S26/3, P94/1, and W73 (330, 390, and 475 bp, respectively). Two bands were observed in reaction mixtures containing DNA from two strains and one band was detected in reaction mixtures containing DNA from one strain. In order to assess the
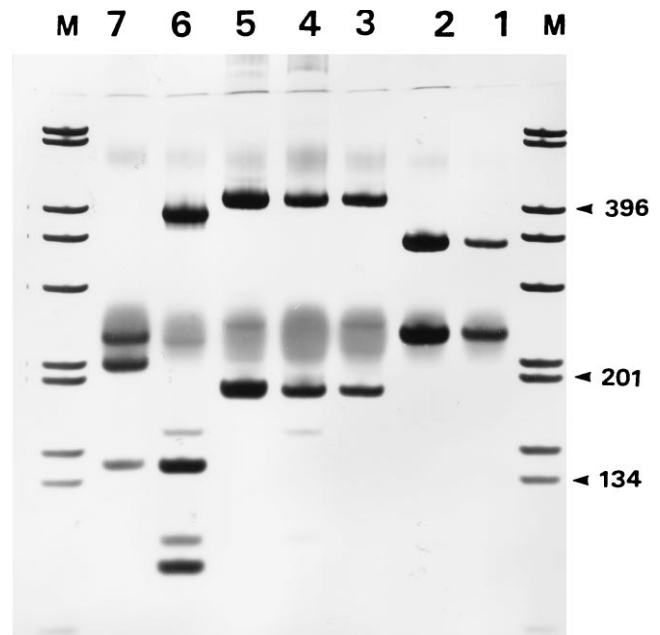

FIG. 3. *Alu*I RE analysis of DNA amplified using PCR-D1 and PCR-D2 and resolved on a 10% polyacrylamide gel. Lane 1, S26/3; lane 2, C94/1; lane 3, W73; lane 4, P787; lane 5, E58; lane 6, GPIC; lane 7, P94/1; lanes M, 1 Kb DNA size markers (Gibco BRL). Numbers on the right are in base pairs.
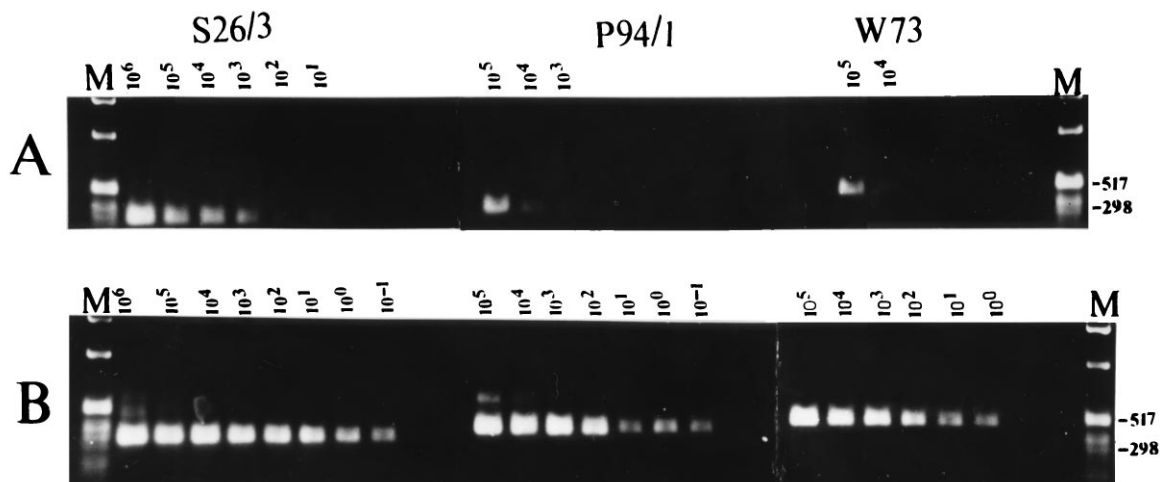
FIG. 4. Sensitivities of PCRs using specific *C. psittaci* and *C. pecorum* primers and PCR-D2. Amplifications of dilutions by using PCR-Ab, PCR-Av, PCR-Pe, and PCR-D2 in single reactions (A) and by using nested reactions (B). For the latter, DNA samples were initially amplified with PCR-D1 and PCR-D2 and then were reamplified with the specific primers and PCR-D2. Numbers to the right of each panel are molecular sizes (in base pairs).

wider applicability of this typing system, multiplex PCRs were carried out with DNAs extracted from both *C. psittaci* and *C. pecorum* strains of bovine, ovine, and avian origins (Fig. 5B). Single DNA bands of the correct size were obtained in all cases except for ZC53, which appears to contain a mixture of *C. pecorum* and *C. psittaci* (abortion strain). The *Alu*I digest of DNA amplified from this strain also exhibited both *C. psittaci* and *C. pecorum* profiles (data not shown).

DISCUSSION

In this study, partial *Omp2* nucleotide sequences and predicted amino acid sequences of representative *C. psittaci* (abortion and pigeon) and *C. pecorum* (enteric, encephalomyelitis, and polyarthritis) strains were aligned. In general, *C. psittaci* strains demonstrate approximately 72% nucleotide and 65% amino acid identity to *C. pecorum* strains. *C. psittaci* abortion strains showed identical nucleotide sequences and greater-than-95% similarity to the avian strains 6BC, A22/M, and P94/1. *C. pecorum* enteric, polyarthritis, and encephalomyelitis strains show greater-than-99% identity.

Intraspecies conservation of sequences within this region of *Omp2* was not entirely unexpected, given the RE results obtained in this study and in other studies. *Alu*I RE analyses of DNA amplified from this region indicate a distinct pattern for *C. psittaci* abortion and pigeon and *C. pecorum* strains, which is in agreement with the nucleotide sequences obtained for these strains. Using different REs, Watson et al. (36) grouped all *C. psittaci* abortion strains and *C. pecorum* P787 into distinct groups. The lack of variability observed in this region of the *Omp2* gene of *C. pecorum* strains is in sharp contrast to the high levels of variability detected in the MOMP gene of these strains, for which at least 13 separate *Alu*I profiles have been described (2, 8).

The simultaneous detection and typing of *C. psittaci* ovine abortion and *C. pecorum* strains in placental or fecal material offer an attractive alternative to some of the current nucleic acid-based detection systems, which generally involve PCR amplification followed by RE analysis of amplified DNA in conjunction with polyacrylamide gel electrophoresis or agarose gel electrophoresis (2, 8, 36). Serovar-specific MOMP gene primers have been developed to differentiate mixed *C. tracho-*
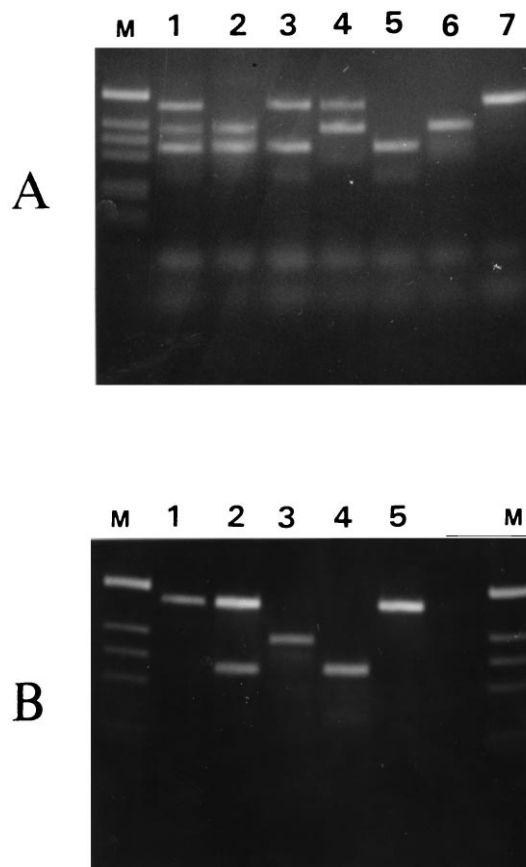
FIG. 5. Specificities of multiplex PCRs using PCR-Ab, PCR-Av, PCR-Pe, and PCR-D2. (A) Amplification of DNA samples containing mixtures of S26/3, P94/1, and W73. Lane 1, S26/3, P94/1, and W73; lane 2, P94/1 and S26/3; lane 3, W73 and S26/3; lane 4, P94/1 and W73; lane 5, S26/3 alone; lane 6, P94/1 alone; lane 7, W73 alone; lane M, 1 Kb DNA size markers (Gibco BRL). (B) Amplification of bovine and ovine strains of chlamydia by using the specific primers and PCR-D2. Lane 1, C95/38; lane 2, ZC53; lane 3, VF88/2122; lane 4, LV350/93; lane 5, PV3056/3; lanes M, 1 Kb DNA size markers.

matis strains in clinical samples (7, 21). Multiplex PCRs using species-specific primers have also been developed to detect and differentiate *C. trachomatis* and *C. pneumoniae* strains in respiratory tract specimens (23).

In the present study, sensitivity experiments using DNA extracted from artificially infected placental or fecal material were carried out. The failure to amplify DNA purified from fecal samples by the standard phenol-chloroform-isoamyl alcohol method could be due to the presence of PCR inhibitors. Similar problems were also reported in other studies involved in the extraction of DNA, for PCR purposes, from human stool samples (20). Heme molecules and their derivatives, acidic polysaccharides, have been recognized as possible PCR inhibitors in other studies (14, 17). The use of cetyltrimethylammonium bromide following proteinase K treatment in DNA extraction procedures was reported to significantly reduce the presence of PCR inhibitors and incidence of false-negative results in DNA extracted from bronchoalveolar lavage fluid (22). In this study, PCR inhibitors present in animal feces were eliminated with a commercial DNA purification system, in which the DNA to be isolated binds to glass beads in the presence of sodium iodide and is subsequently washed and eluted. The fecal strain ZC53 was found to contain both *C. psittaci* (abortion) and *C. pecorum* genotypes. This fecal strain was originally isolated from a flock that had a previous history of abortion (18). Laboratory contamination of this sample cannot be ruled out but is thought to be very unlikely. The occurrence of mixed infections with *C. psittaci* and *C. pecorum* is consistent with previous reports of excretion of such strains from the gut, particularly in flocks which have experienced abortions (2, 3, 28). Using the PCR detection system described in this report to investigate the prevalence of mixed infections in large numbers of fecal samples could help increase our understanding of the role of such infections in chlamydial pathogenesis.

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