US005348857A

United States Patent [19]

Ficht et al.

[11] Patent Number: 5,348,857

[45] Date of Patent: Sep. 20, 1994

[54] **PROBES AND METHOD FOR IDENTIFYING SPECIES AND BIOVARS OF BRUCELLA**

[75] Inventors: **Thomas A. Ficht; L. Garry Adams,** both of College Station, Tex.

[73] Assignee: **Texas A & M University,** College Station, Tex.

[21] Appl. No.: **972,791**

[22] Filed: **Nov. 6, 1992**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 527,017, May 22, 1990.

[51] **Int. Cl.**[5] **C12P 19/34**

[52] **U.S. Cl.** **435/6;** 435/91.2; 435/29; 435/34; 436/501; 436/811; 536/23.7; 935/77; 935/78

[58] **Field of Search** 435/6, 9, 1.2, 29, 34; 436/501, 811; 536/23.7; 935/77, 78

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,358,535	3/1982	Falkow et al.	435/5
4,683,202	7/1987	Mullis	435/91

OTHER PUBLICATIONS

Nicoletti, *Am. J. Vet. Res.,* (1981) 42:1494–1497.
Farrell, *Res. Vet. Sci.,* (1974) 16:280–286.
Ficht et al., *Mol. Microbiol.,* (1990) 4:1135–1142.
Ficht et al., *Infect. Immunol.,* (1988) 56:2036–2046.
Heck et al., *Am. J. Vet. Res.,* (1980) 41:2082–2084.
Huber et al., *Am. J. Vet. Res.,* (1986) 47:1529–1531.
Saiki et al., *Science,* (1988) 239:487–491.
Timbs et al., *N.Z. vet. J.,* (1978) 26:52–56.
Embase Abstract No. 89259898, Ficht et al., Infect. Immun. (USA), 1989, (3281–3291).
Hudson et al., J. Miol. Bio., (1984) 180:1023–1051.
Raitio et al., The EMBO Journal, 6:2825–2833 (1987).

Primary Examiner—Amelia Burgess Yarbrough
Attorney, Agent, or Firm—Pravel, Hewitt, Kimball & Krieger

[57] **ABSTRACT**

A method for detecting Brucella infection in an animal which is reliable, rapid, and able to identify species and biovars of Brucella. The detection method includes the amplification of the omp2 gene locus of Brucella and analysis of restriction digestion fragments specific to Brucella and to individual species and groups of biovars of Brucella.

13 Claims, 26 Drawing Sheets

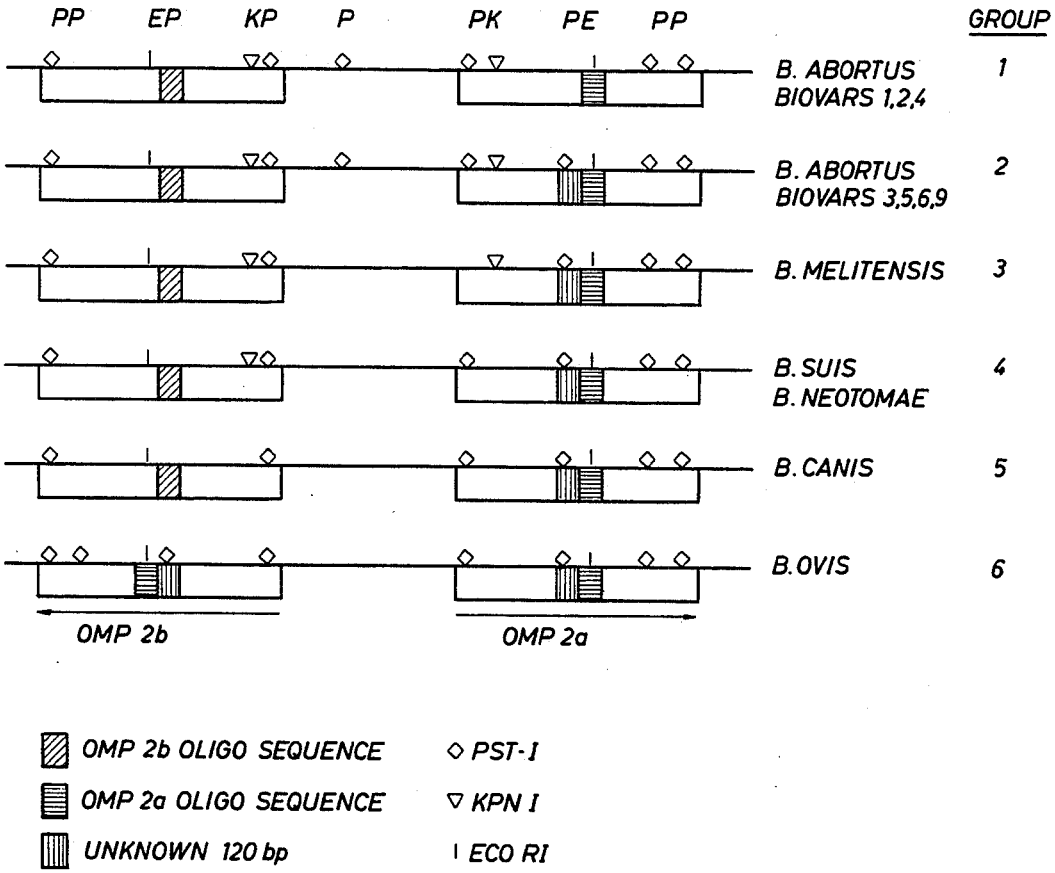
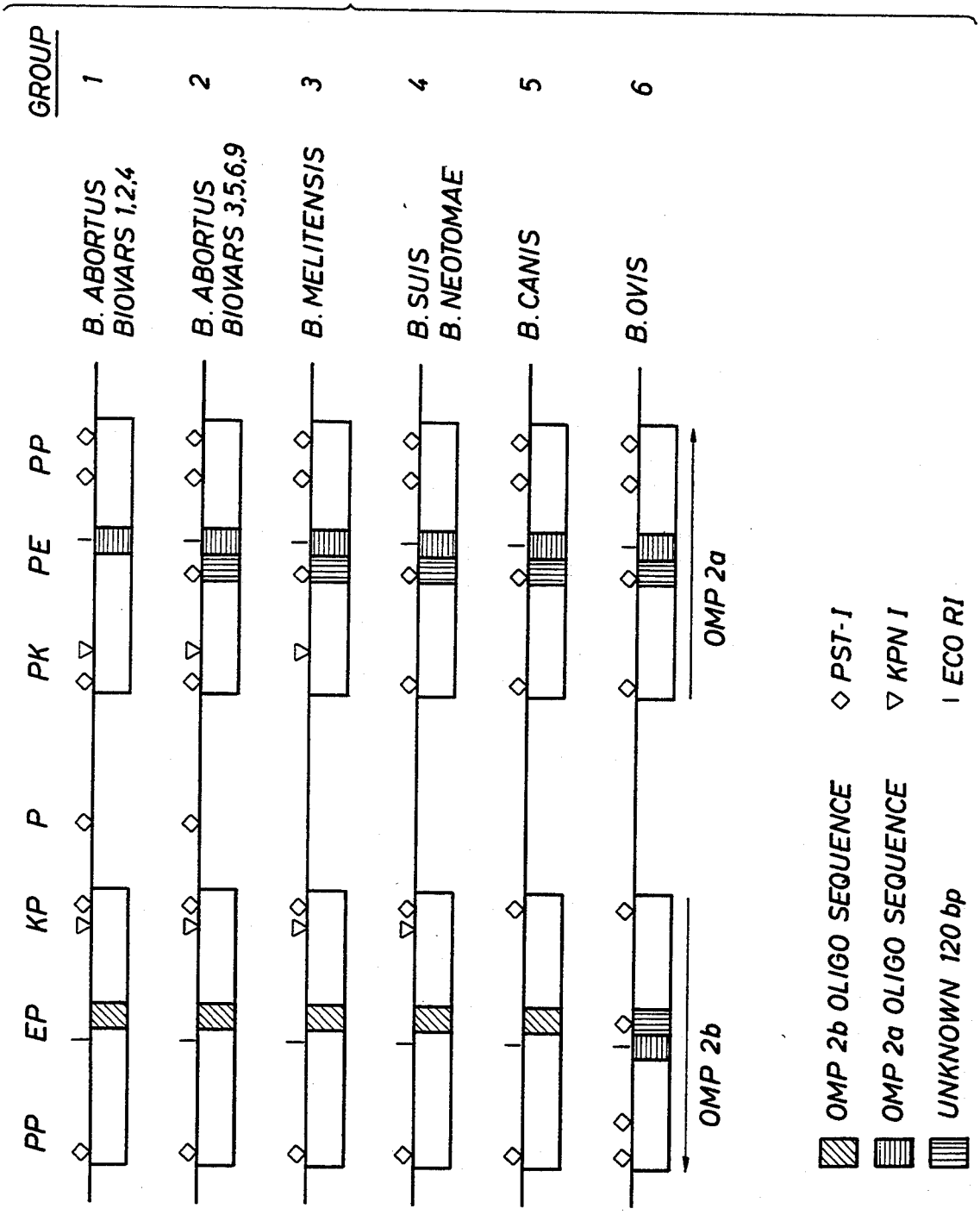


FIG. 1


GROUP
1
2
3
4
5
6
B. ABORTUS BIOVARS 1,2,4
B. ABORTUS BIOVARS 3,5,6,9
B. MELITENSIS
B. SUIS B. NEOTOMAE
B. CANIS
B. OVIS
PP EP KP P PK PE PP
OMP 2b
OMP 2a
OMP 2b OLIGO SEQUENCE
OMP 2a OLIGO SEQUENCE
UNKNOWN 120 bp
◇ PST-1
▽ KPN I
| ECO RI

SPECIES ALIGNMENT FORMATTED ALIGNMENT

```
CONCENSUS          CAGGCGATCT TCCGCGACCC CTGTAGAAAG ACTGCGGTCA GCATAAAAAG CAAGCATCTG   60

B.OVIS 1ST OMP II  .......... .......... .......... .......... .......... ..........  60
B.SUIS OMP II      .......... .......... .......... .......... .......... ..........  60
B.B5 OMP II        .......... .......... .......... .......... .......... ..........  60
B.MELIT 1ST OMP II .......... .......... .......... .......... .......... ..........  59
B.CANIS OMP II     .......... .......... .......... .......... .......... ..........  60
B.NEOTOMAE OMP II  .......... .......... .......... .......... .......... ..........  60
B.2308 OMP II      .......... .......... .......... .......... .......... ..........  60

CONCENSUS          ATGCTGCACG AGGGCAACAA AAAACCCGGY ATTTCTGCCG GGTTTCTGTA TCCAATCCGT  120

B.OVIS 1ST OMP II  .......... .......... .........C .......... .......... ..........  120
B.SUIS OMP II      .......... .......... .........T .......... .......... ..........  120
B.B5 OMP II        .......... .......... .........C .......... .......... ..........  120
B.MELIT 1ST OMP II .......... .......... .........C .......... .......... ..........  119
B.CANIS OMP II     .......... .......... .........T .......... .......... ..........  120
B.NEOTOMAE OMP II  .......... .......... .........C .......... .......... ..........  120
B.2308 OMP II      .......... .......... .........C .......... .......... ..........  120

CONCENSUS          AATGGATTAG AACGAACGCT GGAAGCGAAC GATACCGCCC CAAGCATTGT CTTCAGCAAC  180

B.OVIS 1ST OMP II  .......... .......... .......... .......... .......... ..........  180
B.SUIS OMP II      .......... .......... .......... .......... .......... ..........  180
B.B5 OMP II        .......... .......... .......... .......... .......... ..........  180
B.MELIT 1ST OMP II .......... .......... .......... .......... .......... ..........  179
B.CANIS OMP II     .......... .......... .......... .......... .......... ..........  180
B.NEOTOMAE OMP II  .......... .......... .......... .......... .......... ..........  180
B.2308 OMP II      .......... .......... .......... .......... .......... ..........  180

CONCENSUS          RGTGTTCTTC CACTCGCCAC CAAACTTGGT GTAGGAAACT TCCGGMGYAA CGGTGAAGCC  240

B.OVIS 1ST OMP II  G......... .......... .......... .......... .....C.T.. ..........  240
B.SUIS OMP II      G......... .......... .......... .......... .....C.T.. ..........  240
B.B5 OMP II        G......... .......... .......... .......... .....C.T.. ..........  240
B.MELIT 1ST OMP II A......... .......... .......... .......... .....C.T.. ..........  239
B.CANIS OMP II     G......... .......... .......... .......... .....C.T.. ..........  240
B.NEOTOMAE OMP II  G......... .......... .......... .......... .....A.C.. ..........  240
B.2308 OMP II      G......... .......... .......... .......... .....C.T.. ..........  240
```

FIG. 2 A

```
CONCENSUS              AGGAACCAGT TCGTAAGCAA CGTTAGCCGT AACTGCCGTC TTGCCCCAGT CGTCATGCGC   300

B.OVIS 1ST OMP II      .......... .......... .......... .......... .......... ..........   300
B.SUIS OMP II          .......... .......... .......... .......... .......... ..........   300
B.B5 OMP II            .......... .......... .......... .......... .......... ..........   300
B.MELIT 1ST OMP II     .......... .......... .......... .......... .......... ..........   299
B.CANIS OMP II         .......... .......... .......... .......... .......... ..........   300
B.NEOTOMAE OMP II      .......... .......... .......... .......... .......... ..........   300
B.2308 OMP II          .......... .......... .......... .......... .......... ..........   300

CONCENSUS              AGCCTGCAGR TTGAAGGYWG CCTTYTSSGT RGCMWKRWAC TTYRSACCAC CCCAGACAGC   360

B.OVIS 1ST OMP II      .........G .......CA. ....C.GC.. A..CTGAT.. ..CAG..... ..........   360
B.SUIS OMP II          .........G .......CA. ....C.GC.. A..CTGAT.. ..CAG..... ..........   360
B.B5 OMP II            .........G .......CA. ....C.GC.. A..CTGAT.. ..CAG..... ..........   360
B.MELIT 1ST OMP II     .........G .......CA. ....C.GC.. A..CTGAT.. ..CAG..... ..........   359
B.CANIS OMP II         .........G .......CA. ....C.GC.. A..CTGAT.. ..CAG..... ..........   360
B.NEOTOMAE OMP II      .........A .......TT. ....T.CG.. G..AATGA.. ..TGC..... ..........   360
B.2308 OMP II          .........G .......CA. ....C.GC.. A..CTGAT.. ..CAG..... ..........   360

CONCENSUS              CCAATCGCCG CCCCACTGRC CGTAGTTCTG RTYCGGCGTM GCWGCGGACG AATATGCGCC   420

B.OVIS 1ST OMP II      .......... ........A. .......... G.T......C ..T....... ..........   420
B.SUIS OMP II          .......... ........G. .......... A.C......A ..A....... ..........   420
B.B5 OMP II            .......... ........G. .......... A.C......A ..A....... ..........   420
B.MELIT 1ST OMP II     .......... ........G. .......... A.C......A ..A....... ..........   419
B.CANIS OMP II         .......... ........G. .......... A.C......A ..A....... ..........   420
B.NEOTOMAE OMP II      .......... ........A. .......... G.T......C ..T....... ..........   420
B.2308 OMP II          .......... ........G. .......... A.C......A ..A....... ..........   420

CONCENSUS              CTGCARCCAW ACCGAGAACY GGTCGGTGAT GTTGACGTCG CCACGAACCT TKGYAGCCCA   480

B.OVIS 1ST OMP II      .....G...T .........C .......... .......... .......... .T.T......   480
B.SUIS OMP II          .....A...A .........T .......... .......... .......... .G.C......   480
B.B5 OMP II            .....A...A .........T .......... .......... .......... .G.C......   480
B.MELIT 1ST OMP II     .....A...A .........T .......... .......... .......... .G.C......   479
B.CANIS OMP II         .....A...A .........T .......... .......... .......... .G.C......   480
B.NEOTOMAE OMP II      .....A...A .........T .......... .......... .......... .G.C......   480
B.2308 OMP II          .....A...A .........T .......... .......... .......... .G.C......   480
```

FIG. 2 B

```
CONCENSUS              TTCTTCKATG ACCGAGTCAT AGGCAACAAC ACCAGCGATC GAACCCCAGC CGCCAGCATA   540

B.OVIS 1ST OMP II      ......G... .......... .......... .......... .......... ..........   540
B.SUIS OMP II          ......G... .......... .......... .......... .......... ..........   540
B.B5 OMP II            ......G... .......... .......... .......... .......... ..........   540
B.MELIT 1ST OMP II     ......G... .......... .......... .......... .......... ..........   539
B.CANIS OMP II         ......G... .......... .......... .......... .......... ..........   540
B.NEOTOMAE OMP II      ......G... .......... .......... .......... .......... ..........   540
B.2308 OMP II          ......T... .......... .......... .......... .......... ..........   540

CONSENSUS              YTTCAGGCCG CCAACAACGT SMGGCATGTA RCCGTCGATS BKGTARTYGG TCGTGCCAGT   600

B.OVIS 1ST OMP II      T......... .......... GC........ A........C GT...A.C.- ----------   589
B.SUIS OMP II          C......... .......... CA........ G........G CG...G.T.. ..........   600
B.B5 OMP II            C......... .......... CA........ G........G TG...G.T.. ..........   600
B.MELIT 1ST OMP II     C......... .......... CA........ G........G TG...G.T.. ..........   599
B.CANIS OMP II         C......... .......... CA........ G........G TG...G.T.. ..........   600
B.NEOTOMAE OMP II      C......... .......... CA........ G........G TG...G.T.. ..........   600
B.2308 OMP II          C......... .......... CA........ G........G TG...G.T.. ..........   600

CONSENSUS              GTAAYYRYCR WCGTYKTCGC CACCCTGTTC GAGAGCGATC ACAGCCGAGA AGCCGTTTCC   660

B.OVIS 1ST OMP II      ----TTGT.A A...CT.... .......... .......... .......... ..........   645
B.SUIS OMP II          ....CCAC.G T...TG.... .......... .......... .......... ..........   660
B.B5 OMP II            ....CCAC.G T...TG.... .......... .......... .......... ..........   660
B.MELIT 1ST OMP II     ....CCAC.G T...TG.... .......... .......... .......... ..........   659
B.CANIS OMP II         ....CCAC.G T...TG.... .......... .......... .......... ..........   660
B.NEOTOMAE OMP II      ....CCAC.G T...TG.... .......... .......... .......... ..........   660
B.2308 OMP II          ....CCAC.G T...TG.... .......... .......... .......... ..........   660

CONSENSUS              GCCRGTGAAG GTGTASGMGA TCTTGCCGGT GCGGTAGGAG CCAGCCGAGA TCACGTCATC   720

B.OVIS 1ST OMP II      ...G...... .....G.C.. .......... .......... .......... ..........   705
B.SUIS OMP II          ...A...... .....C.A.. .......... .......... .......... ..........   720
B.B5 OMP II            ...A...... .....C.A.. .......... .......... .......... ..........   720
B.MELIT 1ST OMP II     ...A...... .....C.A.. .......... .......... .......... ..........   720
B.CANIS OMP II         ...A...... .....C.A.. .......... .......... .......... ..........   719
B.NEOTOMAE OMP II      ...A...... .....C.A.. .......... .......... .......... ..........   720
B.2308 OMP II          ...A...... .....C.A.. .......... .......... .......... ..........   720
```

FIG. 2 C

```
CONSENSUS            GTTGATGAYA TCRCCGAGGT AACCGGTGAA GGTATGGAAT TCSGATTCRT CGATACCAAC  780

B.OVIS 1ST OMP II    ........T. ..A.....  ..........  ..........  ..G.....G.  ..........  765
B.SUIS OMP II        ........C. ..G.....  ..........  ..........  ..C.....A.  ..........  780
B.B5 OMP II          ........C. ..G.....  ..........  ..........  ..C.....A.  ..........  780
B.MELIT 1ST OMP II   ........C. ..G.....  ..........  ..........  ..C.....A.  ..........  779
B.CANIS OMP II       ........C. ..G.....  ..........  ..........  ..C.....A.  ..........  780
B.NEOTOMAE OMP II    ........C. ..G.....  ..........  ..........  ..C.....A.  ..........  780
B.2308 OMP II        ........C. ..G.....  ..........  ..........  ..C.....A.  ..........  780

CONSENSUS            SYKSARACCA CCRAGCKKGA TATAYGCRAA CTSCAKRWCG GTGCCGSTGC TTACGCTGCC  840

B.OVIS 1ST OMP II    CTTG.A....  ..A...GT..  ....T..A..  ..G..GAT..  ......G...  ..........  825
B.SUIS OMP II        GCGC.G....  ..G...TG..  ....C..G..  ..C..TGA..  ......C...  .----------  831
B.B5 OMP II          GCGC.G....  ..G...TG..  ....C..G..  ..C..TGA..  ......C...  .----------  831
B.MELIT 1ST OMP II   GCGC.G....  ..G...TG..  ....C..G..  ..C..TGA..  ......C...  .----------  830
B.CANIS OMP II       GCGC.G....  ..G...TG..  ....C..G..  ..C..TGA..  ......C...  .----------  831
B.NEOTOMAE OMP II    GCGC.G....  ..G...TG..  ....C..G..  ..C..TGA..  ......C...  .----------  831
B.2308 OMP II        GCGC.G....  ..G...TG..  ....C..G..  ..C..TGA..  ......C...  .----------  831

CONSENSUS            ATCAGCGACA TCACGATCAT CGSTKWMATY RCCRTATTKR CCATCWWSRC SYGAATTGTT  900

B.OVIS 1ST OMP II    ..........  ..........  ..C.GAA..C  G..G....GG  .....ATGA.  GT........  885
B.SUIS OMP II        ----------  ----------  -.G.TTC..T  A..A....TA  .....TACG.  CC........  870
B.B5 OMP II          ----------  ----------  -.G.TTC..T  A..A....TA  .....TACG.  CC........  870
B.MELIT 1ST OMP II   ----------  ----------  -.G.TTC..T  A..A....TA  .....TACG.  CC........  869
B.CANIS OMP II       ----------  ----------  -.G.TTC..T  A..A....TA  .....TACG.  CC........  870
B.NEOTOMAE OMP II    ----------  ----------  -.G.TTC..T  A..A....TA  .....TACG.  CC........  870
B.2308 OMP II        ----------  ----------  -.G.TTC..T  A..A....TA  .....TACG.  CC........  870

CONSENSUS            SSYRGYRTAG TTGAAGCGCA GYKYRGTRWA GGTSYYGAGK GTGCCGAGTT CGGTTTCCGA  960

B.OVIS 1ST OMP II    GCTG.TG...  ..........  .CGTA..AT.  ...GCC...T  ..........  ..........  945
B.SUIS OMP II        CGCA.CA...  ..........  .TTCG..GA.  ...CTT...G  ..........  ..........  930
B.B5 OMP II          CGCA.CA...  ..........  .TTCG..GA.  ...CTT...G  ..........  ..........  930
B.MELIT 1ST OMP II   CGCA.CA...  ..........  .TTCG..GA.  ...CTT...G  ..........  ..........  929
B.CANIS OMP II       CGCA.CA...  ..........  .TTCG..GA.  ...CTT...G  ..........  ..........  930
B.NEOTOMAE OMP II    CGCA.CA...  ..........  .TTCG..GA.  ...CTT...G  ..........  ..........  930
B.2308 OMP II        CGCA.CA...  ..........  .TTCG..GA.  ...CTT...G  ..........  ..........  930
```

FIG. 2 D

```
CONSENSUS           AYYSGTGKWR AMSMKGRRKK SSAAAMSRRS SMYCTTGTCC CAGCCWTTRC GRTCSGWRCC  1020

B.OVIS 1ST OMP II   .TTC...TTG .ACAT.GAGT GC...ACGAG CAC.......  .....T..A. .A..C.AG..  1005
B.SUIS OMP II       .CCG...GAA .CGCG.AGTG CG...CGAGC GCT.......  .....A..G. .G..G.TA..   990
B.B5 OMP II         .CCG...GAA .CGCG.AGTG CG...CGAGC GCT.......  .....A..G. .G..G.TA..   990
B.MELIT 1ST OMP II  .CCG...GAA .CGCG.AGTG CG...CGAGC GCT.......  .....A..G. .G..G.TA..   990
B.CANIS OMP II      .CCG...GAA .CGCG.AGTG CG...CGAGC GCC.......  .....A..G. .G..G.TA..   989
B.NEOTOMAE OMP II   .CCG...GAA .CGCG.AGTG CG...CGAGC GCT.......  .....T..A. .A..C.AG..   990
B.2308 OMP II       .CCG...GAA .CGCG.AGTG CG...CGAGC GCT.......  .....A..G. .G..G.TA..   990

CONSENSUS           GGWRTAAACG TCRTCGCCGC CCTTTACGTC GTAACGGACG TARCCRYKGA YGCGCAGGCA  1080

B.OVIS 1ST OMP II   ..TA......  ..G.......  ..........  ..........  ..G..GCT..  T.........  1065
B.SUIS OMP II       ..AG......  ..A.......  ..........  ..........  ..A..ATG..  C.........  1050
B.B5 OMP II         ..AG......  ..A.......  ..........  ..........  ..A..ATG..  C.........  1050
B.MELIT 1ST OMP II  ..AG......  ..A.......  ..........  ..........  ..A..ATG..  C.........  1050
B.CANIS OMP II      ..TA......  ..G.......  ..........  ..........  ..A..ATG..  C.........  1049
B.NEOTOMAE OMP II   ..AG......  ..A.......  ..........  ..........  ..A..ATG..  C.........  1050
B.2308 OMP II       ..AG......  ..A.......  ..........  ..........  ..A..ATG..  C.........  1050

CONSENSUS           GGTTTCGGTG CCCGGAATGT AGAAGTAGCC AGCGCCRTAA GCGTCGCAAA CGCGGACATA  1140

B.OVIS 1ST OMP II   ..........  ..........  ..........  ......A...  ..........  ..........  1125
B.SUIS OMP II       ..........  ..........  ..........  ......G...  ..........  ..........  1110
B.B5 OMP II         ..........  ..........  ..........  ......G...  ..........  ..........  1110
B.MELIT 1ST OMP II  ..........  ..........  ..........  ......G...  ..........  ..........  1109
B.CANIS OMP II      ..........  ..........  ..........  ......G...  ..........  ..........  1110
B.NEOTOMAE OMP II   ..........  ..........  ..........  ......G...  ..........  ..........  1110
B.2308 OMP II       ..........  ..........  ..........  ......G...  ..........  ..........  1110

CONSENSUS           TTCAACGGCT TCGGGCTCTG GCGCGACGAT TGCGTCGGCA GCYTGAGCGC CGGAAGCTGC  1200

B.OVIS 1ST OMP II   ..........  ..........  ..........  ..........  ..T.......  ..........  1185
B.SUIS OMP II       ..........  ..........  ..........  ..........  ..C.......  ..........  1170
B.B5 OMP II         ..........  ..........  ..........  ..........  ..C.......  ..........  1170
B.MELIT 1ST OMP II  ..........  ..........  ..........  ..........  ..C.......  ..........  1169
B.CANIS OMP II      ..........  ..........  ..........  ..........  ..C.......  ..........  1170
B.NEOTOMAE OMP II   ..........  ..........  ..........  ..........  ..C.......  ..........  1170
B.2308 OMP II       ..........  ..........  ..........  ..........  ..C.......  ..........  1170
```

FIG. 2 E

```
CONSENSUS            AACCAGAGCT GCAGCGGAGC CAAGGAGAAG GCTCTTGATG TTCATTTCTG ACCTCCAGTC  1260

B.OVIS 1ST OMP II    .......... .......... .......... .......... .......... ..........  1245
B.SUIS OMP II        .......... .......... .......... .......... .......... ..........  1230
B.B5 OMP II          .......... .......... .......... .......... .......... ..........  1230
B.MELIT 1ST OMP II   .......... .......... .......... .......... .......... ..........  1229
B.CANIS OMP II       .......... .......... .......... .......... .......... ..........  1230
B.NEOTOMAE OMP II    .......... .......... .......... .......... .......... ..........  1230
B.2308 OMP II        .......... .......... .......... .......... .......... ..........  1230

CONSENSUS            AAAGTTAAAA ATGGGTCTRG GCATTCTGAT TTGGCTGAAG GACAACCTGT CCCCATCCCC  1320

B.OVIS 1ST OMP II    .........  ........G.  .........  .........  .........  .........  1305
B.SUIS OMP II        .........  ........G.  .........  .........  .........  .........  1290
B.B5 OMP II          .........  ........G.  .........  .........  .........  .........  1290
B.MELIT 1ST OMP II   .........  ........G.  .........  .........  .........  .........  1289
B.CANIS OMP II       .........  ........G.  .........  .........  .........  .........  1290
B.NEOTOMAE OMP II    .........  ........A.  .........  .........  .........  .........  1290
B.2308 OMP II        .........  ........G.  .........  .........  .........  .........  1290

CONSENSUS            TAATTGAAAA AGTCGCCCCG AAGCGCTCCT TCTTCTGAAA GTGAAGATAC TCGCCCATTT  1380

B.OVIS 1ST OMP II    .......... .......... .......... .......... .......... ..........  1365
B.SUIS OMP II        .......... .......... .......... .......... .......... ..........  1350
B.B5 OMP II          .......... .......... .......... .......... .......... ..........  1350
B.MELIT 1ST OMP II   .......... .......... .......... .......... .......... ..........  1349
B.CANIS OMP II       .......... .......... .......... .......... .......... ..........  1350
B.NEOTOMAE OMP II    .......... .......... .......... .......... .......... ..........  1350
B.2308 OMP II        .......... .......... .......... .......... .......... ..........  1350

CONSENSUS            ATTCGTTTCA ACATCGAATA TGTTCTCACA ACCTTTAYGG TGCTGCTATG AAGGGCAGTT  1440

B.OVIS 1ST OMP II    .......... .......... .......... .......C.. .......... ..........  1425
B.SUIS OMP II        .......... .......... .......... .......C.. .......... ..........  1410
B.B5 OMP II          .......... .......... .......... .......T.. .......... ..........  1410
B.MELIT 1ST OMP II   .......... .......... .......... .......C.. .......... ..........  1409
B.CANIS OMP II       .......... .......... .......... .......C.. .......... ..........  1410
B.NEOTOMAE OMP II    .......... .......... .......... .......C.. .......... ..........  1410
B.2308 OMP II        .......... .......... .......... .......T.. .......... ..........  1410
```

FIG. 2 F

```
CONSENSUS            RTTGCWGAAA TGACACRAAA TTACCTGCTT TAGCTCGGCG GATTCATGCT TTATTAACAT  1500

B.OVIS 1ST OMP II    A....A.... ......G... .......... .......... .......... ..........  1485
B.SUIS OMP II        G....T.... ......G... .......... .......... .......... ..........  1470
B.B5 OMP II          G....A.... ......G... .......... .......... .......... ..........  1470
B.MELIT 1ST OMP II   A....A.... ......G... .......... .......... .......... ..........  1469
B.CANIS OMP II       G....T.... ......A... .......... .......... .......... ..........  1470
B.NEOTOMAE OMP II    G....A.... ......G... .......... .......... .......... ..........  1470
B.2308 OMP II        G....A.... ......G... .......... .......... .......... ..........  1470

CONSENSUS            AAGTRAACGC GAATTAACCG ATGTTAACGT TTGAAAATGC AAGTTTTTTA GGATCGCCTR  1560

B.OVIS 1ST OMP II    ....G..... .......... .......... .......... .......... .........G  1545
B.SUIS OMP II        ....G..... .......... .......... .......... .......... .........A  1530
B.B5 OMP II          ....G..... .......... .......... .......... .......... .........G  1530
B.MELIT 1ST OMP II   ....G..... .......... .......... .......... .......... .........G  1530
B.CANIS OMP II       ....A..... .......... .......... .......... .......... .........A  1529
B.NEOTOMAE OMP II    ....G..... .......... .......... .......... .......... .........G  1530
B.2308 OMP II        ....G..... .......... .......... .......... .......... .........G  1530

CONSENSUS            CMGAATAAAG CCGCRRATCT TTCGTCGAAA CAGCCCTTAA CGGAATATGT CGGCAAGGTG  1620

B.OVIS 1ST OMP II    .C........ ....GA.... .......... .......... .......... ..........  1605
B.SUIS OMP II        .C........ ....GA.... .......... .......... .......... ..........  1590
B.B5 OMP II          .A........ ....GA.... .......... .......... .......... ..........  1590
B.MELIT 1ST OMP II   .C........ ....GG.... .......... .......... .......... ..........  1589
B.CANIS OMP II       .C........ ....GA.... .......... .......... .......... ..........  1590
B.NEOTOMAE OMP II    .C........ ....AA.... .......... .......... .......... ..........  1590
B.2308 OMP II        .A........ ....GA.... .......... .......... .......... ..........  1590

CONSENSUS            GCAAGAATCG TCTGAACGGA GAGCAGAAAC CTCGAATCCG TTTCATTTAA TAAGGGCAAG  1680

B.OVIS 1ST OMP II    .......... .......... .......... .......... .......... ..........  1665
B.SUIS OMP II        .......... .......... .......... .......... .......... ..........  1650
B.B5 OMP II          .......... .......... .......... .......... .......... ..........  1650
B.MELIT 1ST OMP II   .......... .......... .......... .......... .......... ..........  1649
B.CANIS OMP II       .......... .......... .......... .......... .......... ..........  1650
B.NEOTOMAE OMP II    .......... .......... .......... .......... .......... ..........  1650
B.2308 OMP II        .......... .......... .......... .......... .......... ..........  1650
```

FIG. 2 G

```
CONSENSUS            TGCGTGCCGG TGCTAAATTG TGGGCCTTTT TAAGCGCGCY ATATATATAA AGAGAATAAT  1740

B.OVIS 1ST OMP II    .......... .......... .......... .........C .......... ..........  1725
B.SUIS OMP II        .......... .......... .......... .........T .......... ..........  1710
B.B5 OMP II          .......... .......... .......... .........C .......... ..........  1710
B.MELIT 1ST OMP II   .......... .......... .......... .........C .......... ..........  1709
B.CANIS OMP II       .......... .......... .......... .........T .......... ..........  1710
B.NEOTOMAE OMP II    .......... .......... .......... .........C .......... ..........  1710
B.2308 OMP II        .......... .......... .......... .........C .......... ..........  1710

CONSENSUS            CCGCAGGAAA TTTTACCAGT TAATGCGTAA ATCGCTTGAA ATGCCCAGGC GTACCGGTTA  1800

B.OVIS 1ST OMP II    .......... .......... .......... .......... .......... ..........  1785
B.SUIS OMP II        .......... .......... .......... .......... .......... ..........  1770
B.B5 OMP II          .......... .......... .......... .......... .......... ..........  1770
B.MELIT 1ST OMP II   .......... .......... .......... .......... .......... ..........  1769
B.CANIS OMP II       .......... .......... .......... .......... .......... ..........  1770
B.NEOTOMAE OMP II    .......... .......... .......... .......... .......... ..........  1770
B.2308 OMP II        .......... .......... .......... .......... .......... ..........  1770

CONSENSUS            TCTCGCCTTT ACCGGAGAGG TGGCCGAGTG GTCGAAGGCG CTCCCCTGCT AAGGGAGTAG  1860

B.OVIS 1ST OMP II    .......... .......... .......... .......... .......... ..........  1845
B.SUIS OMP II        .......... .......... .......... .......... .......... ..........  1830
B.B5 OMP II          .......... .......... .......... .......... .......... ..........  1830
B.MELIT 1ST OMP II   .......... .......... .......... .......... .......... ..........  1829
B.CANIS OMP II       .......... .......... .......... .......... .......... ..........  1830
B.NEOTOMAE OMP II    .......... .......... .......... .......... .......... ..........  1830
B.2308 OMP II        .......... .......... .......... .......... .......... ..........  1830

CONSENSUS            ACCTCAAAAG GGTCTCGTGG GTTCGAATCC CATCCTCTCC GCCAGTTTTT CCAATATCCC  1920

B.OVIS 1ST OMP II    .......... .......... .......... .......... .......... ..........  1905
B.SUIS OMP II        .......... .......... .......... .......... .......... ..........  1890
B.B5 OMP II          .......... .......... .......... .......... .......... ..........  1890
B.MELIT 1ST OMP II   .......... .......... .......... .......... .......... ..........  1889
B.CANIS OMP II       .......... .......... .......... .......... .......... ..........  1890
B.NEOTOMAE OMP II    .......... .......... .......... .......... .......... ..........  1890
B.2308 OMP II        .......... .......... .......... .......... .......... ..........  1890
```

FIG. 2 H

```
CONSENSUS           AGCAAATCTT TATGTGTTCG ACGCGCTTGA TTTCATACGG AATCGGCTTT TACCCCTCGC  1980

B.OVIS 1ST OMP II   .......... .......... .......... .......... .......... ..........  1965
B.SUIS OMP II       .......... .......... .......... .......... .......... ..........  1950
B.B5 OMP II         .......... .......... .......... .......... .......... ..........  1950
B.MELIT 1ST OMP II  .......... .......... .......... .......... .......... ..........  1949
B.CANIS OMP II      .......... .......... .......... .......... .......... ..........  1950
B.NEOTOMAE OMP II   .......... .......... .......... .......... .......... ..........  1950
B.2308 OMP II       .......... .......... .......... .......... .......... ..........  1950

CONSENSUS           GCACTGAATC TCTGTTTTTC CAGGCTACGA ATCCAGAAAA CAAGCAAGCC ATTGATAAGT  2040

B.OVIS 1ST OMP II   .......... .......... .......... .......... .......... ..........  2025
B.SUIS OMP II       .......... .......... .......... .......... .......... ..........  2010
B.B5 OMP II         .......... .......... .......... .......... .......... ..........  2010
B.MELIT 1ST OMP II  .......... .......... .......... .......... .......... ..........  2009
B.CANIS OMP II      .......... .......... .......... .......... .......... ..........  2010
B.NEOTOMAE OMP II   .......... .......... .......... .......... .......... ..........  2010
B.2308 OMP II       .......... .......... .......... .......... .......... ..........  2010

CONSENSUS           AATGGCTATT CAAAATTCTG GCRATTCTTG ACTGGAGGTC AGAAATGAAC ATCAAGAGCC  2100

B.OVIS 1ST OMP II   .......... .......... ..A....... .......... .......... ..........  2085
B.SUIS OMP II       .......... .......... ..G....... .......... .......... ..........  2070
B.B5 OMP II         .......... .......... ..G....... .......... .......... ..........  2070
B.MELIT 1ST OMP II  .......... .......... ..G....... .......... .......... ..........  2069
B.CANIS OMP II      .......... .......... ..G....... .......... .......... ..........  2070
B.NEOTOMAE OMP II   .......... .......... ..G....... .......... .......... ..........  2070
B.2308 OMP II       .......... .......... ..G....... .......... .......... ..........  2070

CONSENSUS           TTCTCCTTGG CTCCGCYGCA GCTCTGGTTG CAGCTTCCGG CGCTCARGCT GCCGACGCAA  2160

B.OVIS 1ST OMP II   .......... ......T... .......... .......... ......A... ..........  2145
B.SUIS OMP II       .......... ......T... .......... .......... ......G... ..........  2130
B.B5 OMP II         .......... ......T... .......... .......... ......G... ..........  2130
B.MELIT 1ST OMP II  .......... ......C... .......... .......... ......G... ..........  2129
B.CANIS OMP II      .......... ......T... .......... .......... ......G... ..........  2130
B.NEOTOMAE OMP II   .......... ......T... .......... .......... ......G... ..........  2130
B.2308 OMP II       .......... ......T... .......... .......... ......G... ..........  2130
```

FIG. 2 I

```
CONSENSUS           TCGTCGCGCC RGAGCCCGAA GCCGTTGAAT ATGTCCGCGT TTGCGACGCT TAYGGCGCTG  2220

B.OVIS 1ST OMP II   .......... A......... .......... .......... .......... ..T.......  2205
B.SUIS OMP II       .......... A......... .......... .......... .......... ..C.......  2190
B.B5 OMP II         .......... A......... .......... .......... .......... ..C.......  2190
B.MELIT 1ST OMP II  .......... A......... .......... .......... .......... ..C.......  2189
B.CANIS OMP II      .......... A......... .......... .......... .......... ..C.......  2190
B.NEOTOMAE OMP II   .......... G......... .......... .......... .......... ..C.......  2190
B.2308 OMP II       .......... A......... .......... .......... .......... ..C.......  2190

CONSENSUS           GCTACTTCTA CATTCCGGGC ACCGAAACCT GCCTGCGCRT CMRYGGYTAC GTCCGTTACG  2280

B.OVIS 1ST OMP II   .......... .......... .......... ........A. .AGC..C... ..........  2265
B.SUIS OMP II       .......... .......... .......... ........G. .CAT..T... ..........  2250
B.B5 OMP II         .......... .......... .......... ........G. .CAT..T... ..........  2250
B.MELIT 1ST OMP II  .......... .......... .......... ........G. .CAT..T... ..........  2249
B.CANIS OMP II      .......... .......... .......... ........G. .CAT..T... ..........  2250
B.NEOTOMAE OMP II   .......... .......... .......... ........A. .AGC..C... ..........  2250
B.2308 OMP II       .......... .......... .......... ........G. .CAT..T... ..........  2250

CONSENSUS           ACGTAAAGGG CGGCGAYGAC GTTTAYWCCG GYWCSGAYCG YAAWGGCTGG GACAAGGGYG  2340

B.OVIS 1ST OMP II   .......... ......C... .....TA... .CT.G..T.. T..A...... ........T.  2325
B.SUIS OMP II       .......... ......C... .....TA... .CT.G..T.. T..A...... ........C.  2310
B.B5 OMP II         .......... ......T... .....CT... .TA.C..C.. C..T...... ........C.  2310
B.MELIT 1ST OMP II  .......... ......T... .....CT... .TA.C..C.. C..T...... ........C.  2309
B.CANIS OMP II      .......... ......C... .....TA... .CT.G..T.. T..A...... ........C.  2310
B.NEOTOMAE OMP II   .......... ......C... .....TA... .CT.G..T.. T..A...... ........C.  2310
B.2308 OMP II       .......... ......T... .....CT... .TA.C..C.. C..T...... ........C.  2310

CONSENSUS           CTCGTTTYGC ACTCATGTTC AACACGAATT CGGAAACCGA ACTCGGCACA CTCGGCACCT  2400

B.OVIS 1ST OMP II   .......T.. .......... .......... .......... .......... ..........  2385
B.SUIS OMP II       .......C.. .......... .......... .......... .......... ..........  2370
B.B5 OMP II         .......C.. .......... .......... .......... .......... ..........  2370
B.MELIT 1ST OMP II  .......C.. .......... .......... .......... .......... ..........  2369
B.CANIS OMP II      .......C.. .......... .......... .......... .......... ..........  2370
B.NEOTOMAE OMP II   .......C.. .......... .......... .......... .......... ..........  2370
B.2308 OMP II       .......C.. .......... .......... .......... .......... ..........  2370
```

FIG. 2 J

```
CONSENSUS           ATACTCAGCT GCGCTTCAAC TACACCAGCA ACAATTCACG TCATGATGGC CAATACGGCG  2460

B.OVIS 1ST OMP II   .......... .......... .......... .......... .......... ..........  2445
B.SUIS OMP II       .......... .......... .......... .......... .......... ..........  2430
B.B5 OMP II         .......... .......... .......... .......... .......... ..........  2430
B.MELIT 1ST OMP II  .......... .......... .......... .......... .......... ..........  2429
B.CANIS OMP II      .......... .......... .......... .......... .......... ..........  2430
B.NEOTOMAE OMP II   .......... .......... .......... .......... .......... ..........  2430
B.2308 OMP II       .......... .......... .......... .......... .......... ..........  2430

CONSENSUS           ATTTCAGCGA TGATCGTGAT GTCGCTGATG GCRGCGTAAN NNNNNNNNNN NNNNNNNNNN  2520

B.OVIS 1ST OMP II   .......... .......... .......... ..R......G CACCGGCACC GATCTGCAGT  2505
B.SUIS OMP II       .......... .......... .......... ..G......G CACCGGCACC GATCTGCAGT  2490
B.B5 OMP II         .......... .......... .......... ..G......G CACCGGCACC GATCTGCAGT  2490
B.MELIT 1ST OMP II  .......... .......... .......... ..G......G CACCGGCACC GATCTGCAGT  2489
B.CANIS OMP II      .......... .......... .......... ..G......G CACCGGCACC GATCTGCAGT  2490
B.NEOTOMAE OMP II   .......... .......... .......... ..G......G CACCGGCACC GATCTGCAGT  2490
B.2308 OMP II       .......... .......... .......... ..G....... .......... ..........  2490

CONSENSUS           NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN  2580

B.OVIS 1ST OMP II   TTGCATATAT CACGCTTGGT GGTTTCAAGG TTGGTATCGA CGAATCCGAA TTCCATACCT  2565
B.SUIS OMP II       TTGCATATAT CACGCTTGGT GGTTTCAAGG TTGGTATCGA CGAATCCGAA TTCCATACCT  2550
B.B5 OMP II         TTGCATATAT CACGCTTGGT GGTTTCAAGG TTGGTATCGA CGAATCCGAA TTCCATACCT  2550
B.MELIT 1ST OMP II  TTGCATATAT CACGCTTGGT GGTTTCAAGG TTGGTATCGA CGAATCCGAA TTCCATACCT  2549
B.CANIS OMP II      TTGCATATAT CACGCTTGGT GGTTTCAAGG TTGGTATCGA CGAATCCGAA TTCCATACCT  2550
B.NEOTOMAE OMP II   TTGCATATAT CACGCTTGGT GGTTTCAAGG TTGGTATCGA CGAATCCGAA TTCCATACCT  2550
B.2308 OMP II       .......... .......... .......... .......... .......... ..........  2550

CONSENSUS           NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNGCA  2640

B.OVIS 1ST OMP II   TCACCGGTTA CCTCGGTGAT ATCATCAACG ATGACGTGAT CTCGGCTGGC TCCTAACC...  2625
B.SUIS OMP II       TCACCGGTTA CCTCGGTGAT GTCATCAACG ATGATGTCGT CGCTGCTGGC TCCTAACC...  2610
B.B5 OMP II         TCACCGGTTA CCTCGGTGAT GTCATCAACG ATGATGTCGT CGCTGCTGGC TCCTAACC...  2610
B.MELIT 1ST OMP II  TCACCGGTTA CCTCGGTGAT GTCATCAACG ATGATGTCGT CGCTGCTGGC TCCTAACC...  2609
B.CANIS OMP II      TCACCGGTTA CCTCGGTGAT GTCATCAACG ATGATGTCGT CGCTGCTGGC TCCTAACC...  2610
B.NEOTOMAE OMP II   TCACCGGTTA CCTCGGTGAT GTCATCAACG ATGATGTCGT CGCTGCTGGC TCCTAACC...  2610
B.2308 OMP II       .......... .......... .......... .......... .......... ..........  2610
```

FIG. 2 K

```
CONSENSUS            CCGGCAAGAT CGCCTACACC TTCACCGGCG GAAACGGCTT CYCGGCTGTG ATCGCTCTCG  2700

B.OVIS 1ST OMP II    .......... .......... .......... .......... .T........ ..........  2685
B.SUIS OMP II        .......... .......... .......... .......... .T........ ..........  2670
B.B5 OMP II          .......... .......... .......... .......... .T........ ..........  2670
B.MELIT 1ST OMP II   .......... .......... .......... .......... .T........ ..........  2669
B.CANIS OMP II       .......... .......... .......... .......... .T........ ..........  2670
B.NEOTOMAE OMP II    .......... .......... .......... .......... .C........ ..........  2670
B.2308 OMP II        .......... .......... .......... .......... .T........ ..........  2670

CONSENSUS            AACAGGGTGG CGAAGACGTT GACAACGATT ACACGATCGA CGGTTACATG CCGCACGTTG  2760

B.OVIS 1ST OMP II    .......... .......... .......... .......... .......... ..........  2745
B.SUIS OMP II        .......... .......... .......... .......... .......... ..........  2730
B.B5 OMP II          .......... .......... .......... .......... .......... ..........  2730
B.MELIT 1ST OMP II   .......... .......... .......... .......... .......... ..........  2729
B.CANIS OMP II       .......... .......... .......... .......... .......... ..........  2730
B.NEOTOMAE OMP II    .......... .......... .......... .......... .......... ..........  2730
B.2308 OMP II        .......... .......... .......... .......... .......... ..........  2730

CONSENSUS            TTGGCGGCCT GAAATATGCT GGCGGCYGGG GTTCGATCGC TGGTGYTGTT GCCTAYGACY  2820

B.OVIS 1ST OMP II    .......... .......... ......T... .......... .....T.... .....T...T  2805
B.SUIS OMP II        .......... .......... ......T... .......... .....C.... .....T...C  2790
B.B5 OMP II          .......... .......... ......T... .......... .....T.... .....T...T  2790
B.MELIT 1ST OMP II   .......... .......... ......C... .......... .....T.... .....T...T  2789
B.CANIS OMP II       .......... .......... ......T... .......... .....T.... .....C...T  2790
B.NEOTOMAE OMP II    .......... .......... ......T... .......... .....T.... .....T...T  2790
B.2308 OMP II        .......... .......... ......T... .......... .....T.... .....T...T  2790

CONSENSUS            CGGTCATCGA AGAATGGGCT ACAAAGGTTC GTGGCGACGT CAACATCACC GACCGGTTCT  2880

B.OVIS 1ST OMP II    .......... .......... .......... .......... .......... ..........  2865
B.SUIS OMP II        .......... .......... .......... .......... .......... ..........  2850
B.B5 OMP II          .......... .......... .......... .......... .......... ..........  2850
B.MELIT 1ST OMP II   .......... .......... .......... .......... .......... ..........  2849
B.CANIS OMP II       .......... .......... .......... .......... .......... ..........  2850
B.NEOTOMAE OMP II    .......... .......... .......... .......... .......... ..........  2850
B.2308 OMP II        .......... .......... .......... .......... .......... ..........  2850
```

FIG. 2 L

```
CONSENSUS          CGGTATGGCT GCAGGGCGCA TATTCGTCCG CAGCGACGCC GAACCAGAAC TACGGTCAGT  2940

B.OVIS 1ST OMP II  .......... .......... .......... .......... .......... ..........  2925
B.SUIS OMP II      .......... .......... .......... .......... .......... ..........  2910
B.B5 OMP II        .......... .......... .......... .......... .......... ..........  2910
B.MELIT 1ST OMP II .......... .......... .......... .......... .......... ..........  2909
B.CANIS OMP II     .......... .......... .......... .......... .......... ..........  2910
B.NEOTOMAE OMP II  .......... .......... .......... .......... .......... ..........  2910
B.2308 OMP II      .......... .......... .......... .......... .......... ..........  2910

CONSENSUS          GGGGCGGCGA TTGGGCTGTC TGGGGTGGTG CAAAGTTCAT TGCCMCCGAA AAGGYAACCT  3000

B.OVIS 1ST OMP II  .......... .......... .......... .......... ....A..... ....C.....  2985
B.SUIS OMP II      .......... .......... .......... .......... ....A..... ....T.....  2970
B.B5 OMP II        .......... .......... .......... .......... ....C..... ....C.....  2970
B.MELIT 1ST OMP II .......... .......... .......... .......... ....C..... ....C.....  2969
B.CANIS OMP II     .......... .......... .......... .......... ....A..... ....C.....  2970
B.NEOTOMAE OMP II  .......... .......... .......... .......... ....A..... ....C.....  2970
B.2308 OMP II      .......... .......... .......... .......... ....C..... ....C.....  2970

CONSENSUS          TCAATCYGCA GGCTGCGCAT GACGACTGGG GCAAGACSGC AGTTACSGCY AACGTYGCTT  3060

B.OVIS 1ST OMP II  ......C... .......... .......... .......C.. ......C..C .....C....  3045
B.SUIS OMP II      ......T... .......... .......... .......C.. ......C..C .....C....  3030
B.B5 OMP II        ......T... .......... .......... .......C.. ......C..C .....C....  3030
B.MELIT 1ST OMP II ......T... .......... .......... .......C.. ......C..C .....C....  3029
B.CANIS OMP II     ......T... .......... .......... .......C.. ......C..C .....C....  3030
B.NEOTOMAE OMP II  ......T... .......... .......... .......G.. ......G..T .....T....  3030
B.2308 OMP II      ......T... .......... .......... .......C.. ......C..C .....C....  3030

CONSENSUS          AYSARCTSGT TCCYGGMTTC ACCRTTACGC CGGAAGTTTC CTACACCAAA TTTGGTGGCG  3120

B.OVIS 1ST OMP II  .TC.G..C.. ...C..A... ...A...... .......... .......... ..........  3105
B.SUIS OMP II      .TC.G..C.. ...C..A... ...A...... .......... .......... ..........  3090
B.B5 OMP II        .TC.G..C.. ...C..A... ...A...... .......... .......... ..........  3090
B.MELIT 1ST OMP II .TC.G..C.. ...C..A... ...A...... .......... .......... ..........  3089
B.CANIS OMP II     .TC.G..C.. ...C..A... ...A...... .......... .......... ..........  3090
B.NEOTOMAE OMP II  .CG.A..G.. ...T..C... ...G...... .......... .......... ..........  3090
B.2308 OMP II      .TC.G..C.. ...C..A... ...A...... .......... .......... ..........  3090
```

FIG. 2 M

```
CONSENSUS            AGTRGAAAGA CACCGTTGCT GAAGACAATG CCTGGGGCGG TATCGTTCGC TTCCAGCGCT  3180

B.OVIS 1ST OMP II    ...A..... .......... .......... .......... .......... ..........  3165
B.SUIS OMP II        ...G..... .......... .......... .......... .......... ..........  3150
B.B5 OMP II          ...G..... .......... .......... .......... .......... ..........  3150
B.MELIT 1ST OMP II   ...A..... .......... .......... .......... .......... ..........  3149
B.CANIS OMP II       ...G..... .......... .......... .......... .......... ..........  3150
B.NEOTOMAE OMP II    ...G..... .......... .......... .......... .......... ..........  3150
B.2308 OMP II        ...G..... .......... .......... .......... .......... ..........  3150

CONSENSUS            CGTTCTAATC AGATCGACGT TAAGCATAGG GCGCCAACGG TTTCCCGTTG GCGCCGGTTC  3240

B.OVIS 1ST OMP II    .......... .......... .......... .......... .......... ..........  3225
B.SUIS OMP II        .......... .......... .......... .......... .......... ..........  3210
B.B5 OMP II          .......... .......... .......... .......... .......... ..........  3210
B.MELIT 1ST OMP II   .......... .......... .......... .......... .......... ..........  3209
B.CANIS OMP II       .......... .......... .......... .......... .......... ..........  3210
B.NEOTOMAE OMP II    .......... .......... .......... .......... .......... ..........  3210
B.2308 OMP II        .......... .......... .......... .......... .......... ..........  3210

CONSENSUS            ATTTGAAACA GCGTTCACGA AAGCGTGAGA ATCGATTCTT CCGGAATGGG GATTCCAGGC  3300

B.OVIS 1ST OMP II    .......... .......... .......... .......... .......... ..........  3285
B.SUIS OMP II        .......... .......... .......... .......... .......... ..........  3270
B.B5 OMP II          .......... .......... .......... .......... .......... ..........  3270
B.MELIT 1ST OMP II   .......... .......... .......... .......... .......... ..........  3269
B.CANIS OMP II       .......... .......... .......... .......... .......... ..........  3270
B.NEOTOMAE OMP II    .......... .......... .......... .......... .......... ..........  3270
B.2308 OMP II        .......... .......... .......... .......... .......... ..........  3270

CONSENSUS            GGATCGACAA TTGAGGGAAT TGCGGGGACG ACAAAAAGCT GGGGGCAACC GGGGGGTCTT  3360

B.OVIS 1ST OMP II    .......... .......... .......... .......... .......... ..........  3345
B.SUIS OMP II        .......... .......... .......... .......... .......... ..........  3330
B.B5 OMP II          .......... .......... .......... .......... .......... ..........  3330
B.MELIT 1ST OMP II   .......... .......... .......... .......... .......... ..........  3329
B.CANIS OMP II       .......... .......... .......... .......... .......... ..........  3330
B.NEOTOMAE OMP II    .......... .......... .......... .......... .......... ..........  3330
B.2308 OMP II        .......... .......... .......... .......... .......... ..........  3330
```

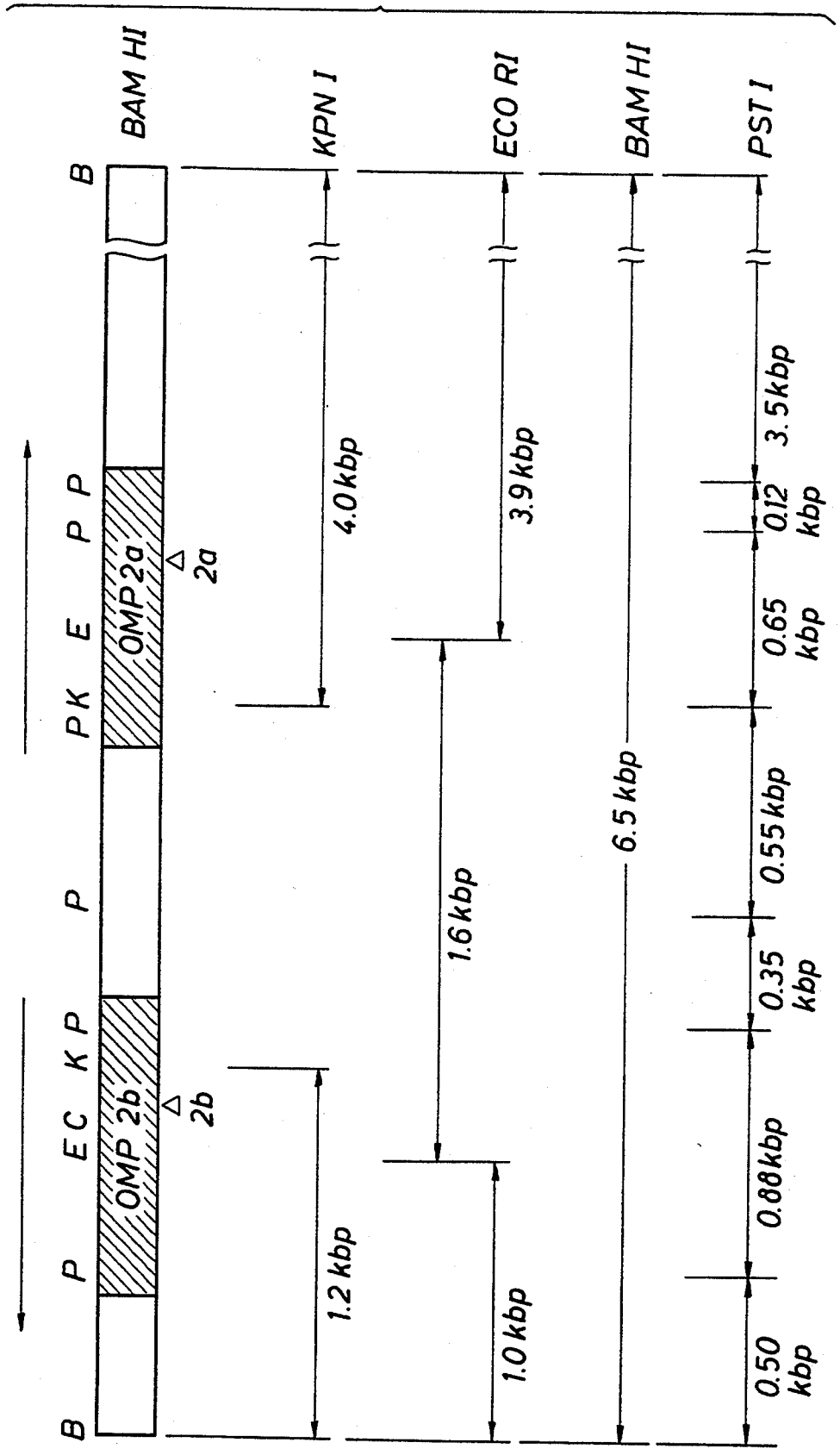
FIG. 2 N

```
CONSENSUS       GTAAAGGATT GAGCCA                    3376

B.OVIS 1ST OMP II  .............. .......           3361
B.SUIS OMP II      .............. .......           3346
B.B5 OMP II        .............. .......           3346
B.MELIT 1ST OMP II .............. .......           3345
B.CANIS OMP II     .............. .......           3346
B.NEOTOMAE OMP II  .............. .......           3346
B.2308 OMP II      .............. .......           3346
```

FIG. 20

FIG. 3


BAM HI
KPN I
ECO RI
BAM HI
PST I
B
P P
P K E
P
E C K P
P
B
OMP 2a
OMP 2b
2a
2b
4.0 kbp
3.9 kbp
1.6 kbp
6.5 kbp
1.2 kbp
1.0 kbp
3.5 kbp
0.12 kbp
0.65 kbp
0.55 kbp
0.35 kbp
0.88 kbp
0.50 kbp

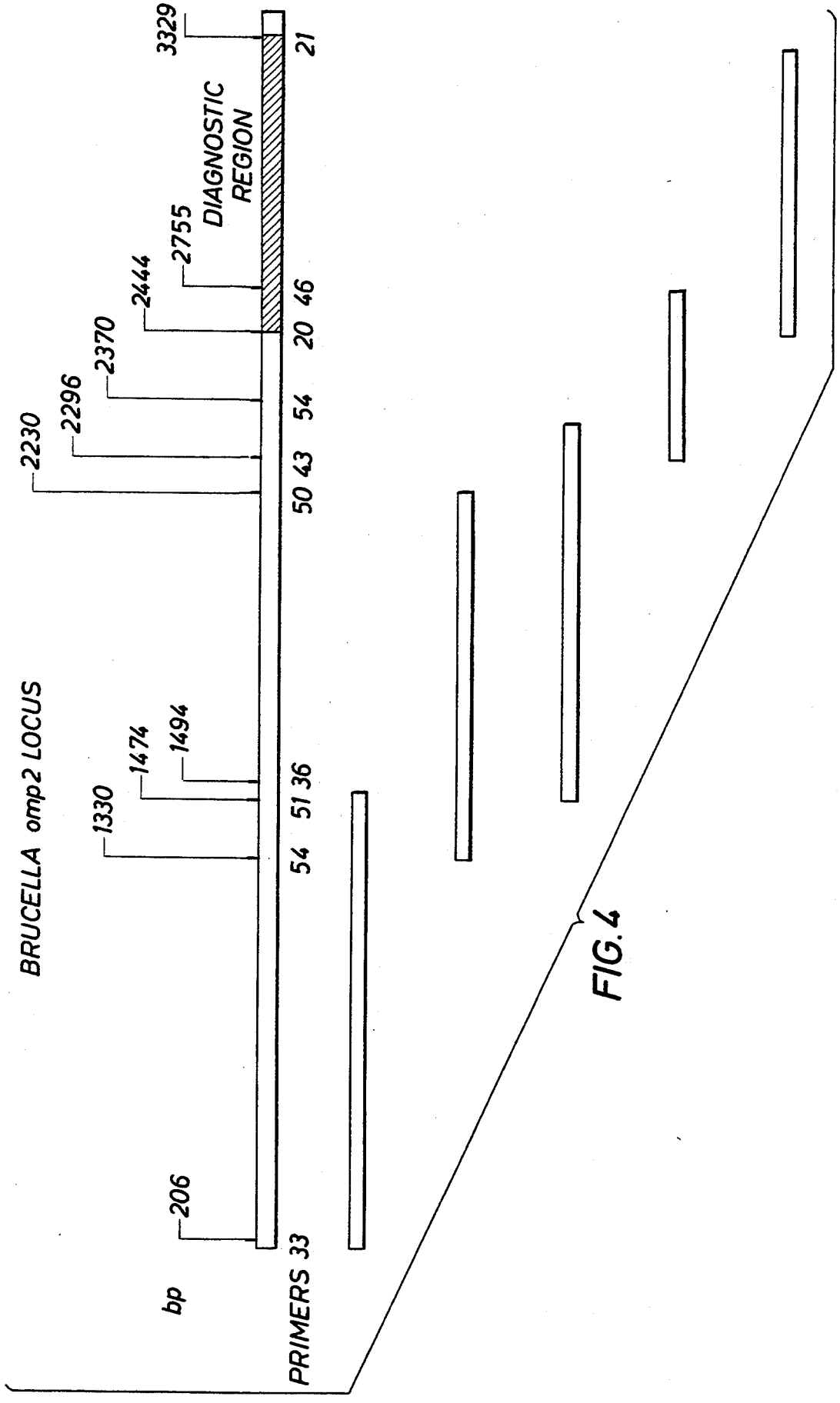

BRUCELLA omp2 LOCUS
bp
206
1330
1474
1494
2230
2296
2370
2444
2755
3329
DIAGNOSTIC REGION
21
20 46
54
50 43
54 51 36
PRIMERS 33

FIG. 4

FIG.5

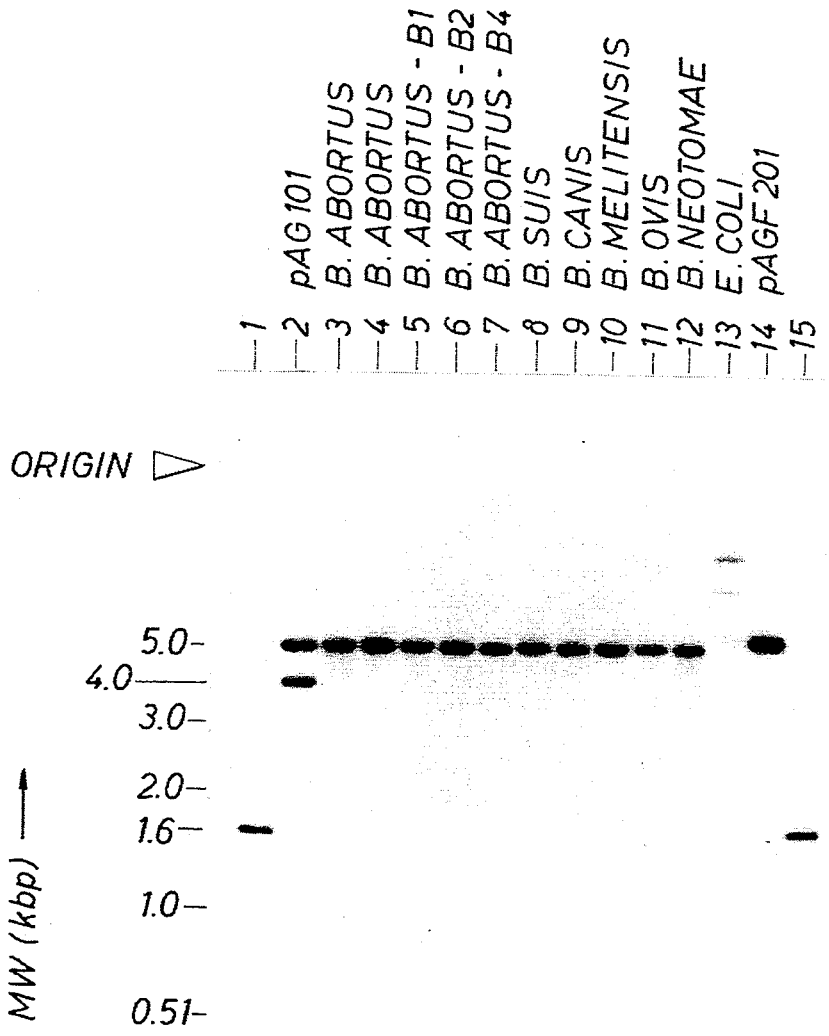

1
2 pAG 101
3 B. ABORTUS
4 B. ABORTUS
5 B. ABORTUS - B1
6 B. ABORTUS - B2
7 B. ABORTUS - B4
8 B. SUIS
9 B. CANIS
10 B. MELITENSIS
11 B. OVIS
12 B. NEOTOMAE
13 E. COLI
14 pAGF 201
15
ORIGIN
5.0
4.0
3.0
2.0
1.6
1.0
0.51
MW (kbp)

FIG.6

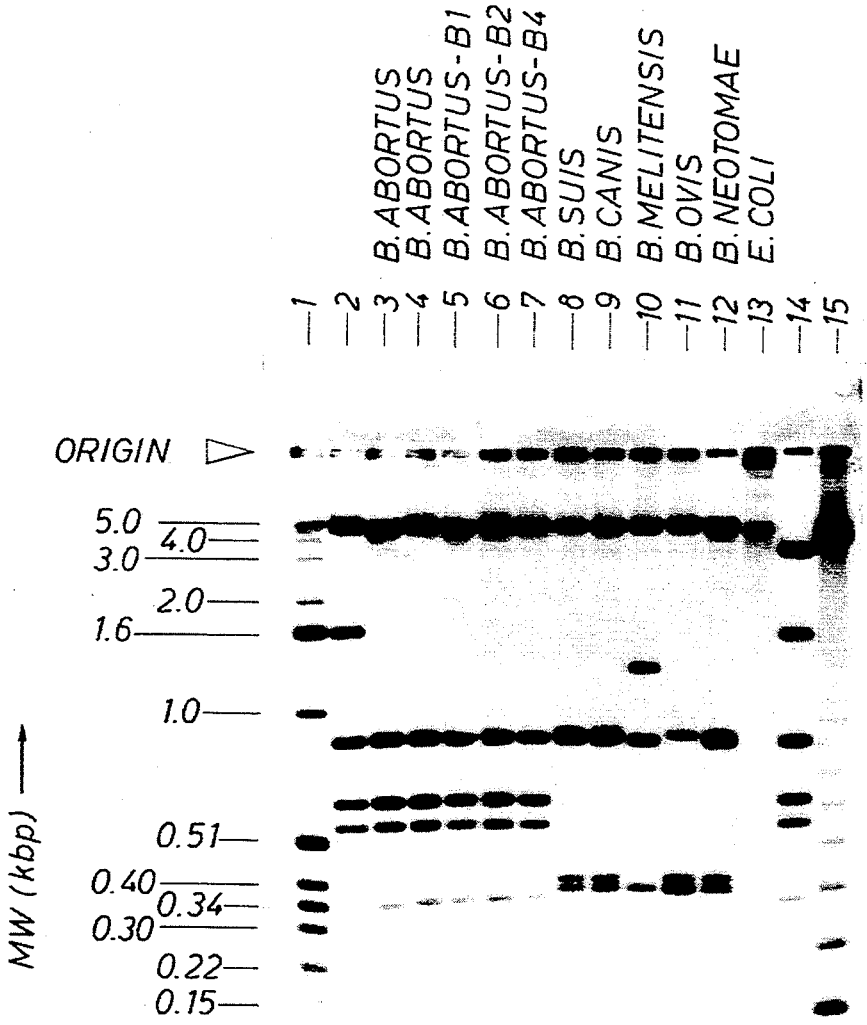

B.ABORTUS
B.ABORTUS
B.ABORTUS-B1
B.ABORTUS-B2
B.ABORTUS-B4
B.SUIS
B.CANIS
B.MELITENSIS
B.OVIS
B.NEOTOMAE
E.COLI
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
ORIGIN
5.0
4.0
3.0
2.0
1.6
1.0
0.51
0.40
0.34
0.30
0.22
0.15
MW (kbp)

FIG. 7

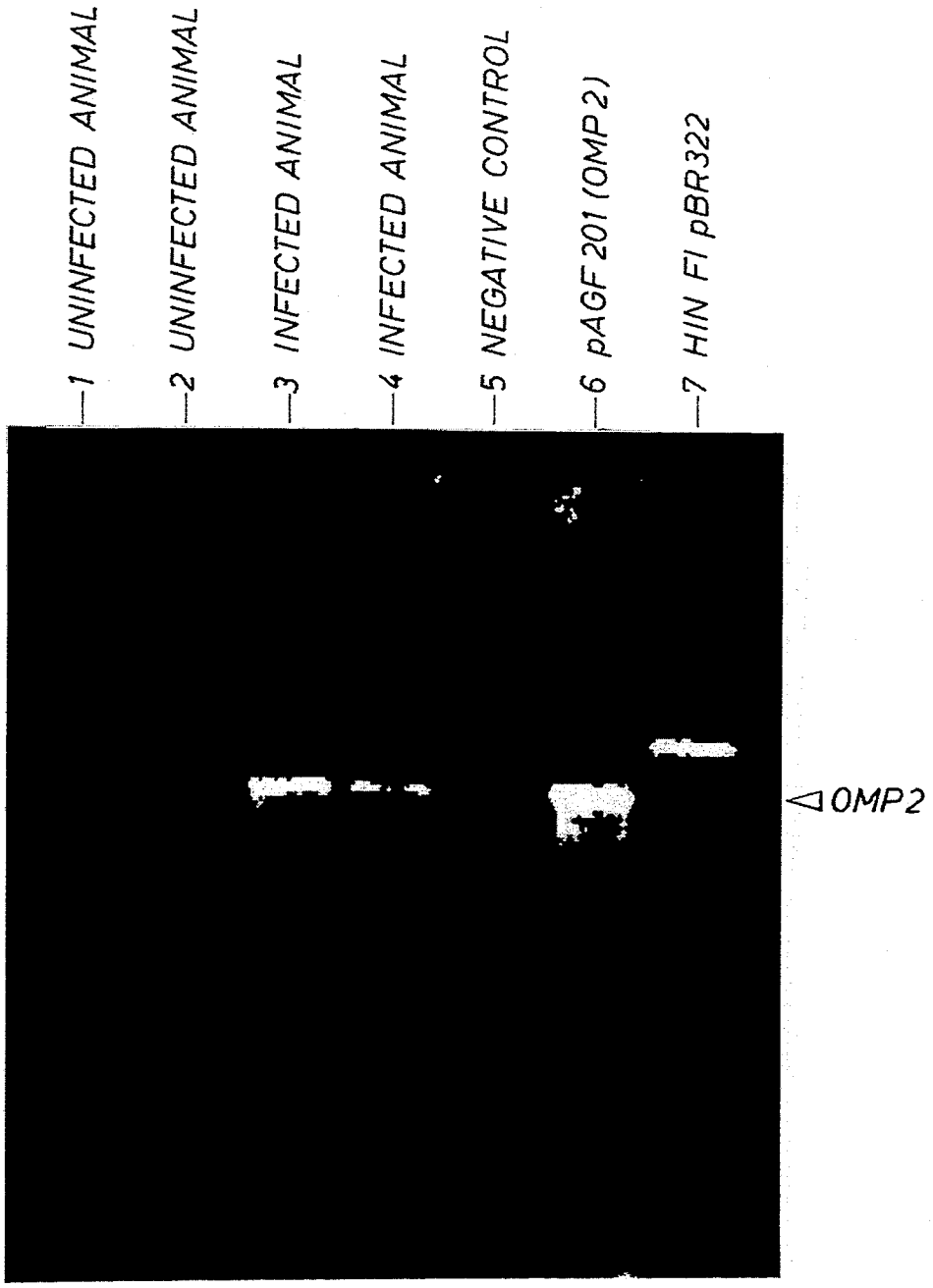

1 UNINFECTED ANIMAL
2 UNINFECTED ANIMAL
3 INFECTED ANIMAL
4 INFECTED ANIMAL
5 NEGATIVE CONTROL
6 pAGF 201 (OMP 2)
7 HIN FI pBR322
OMP2

FIG. 8

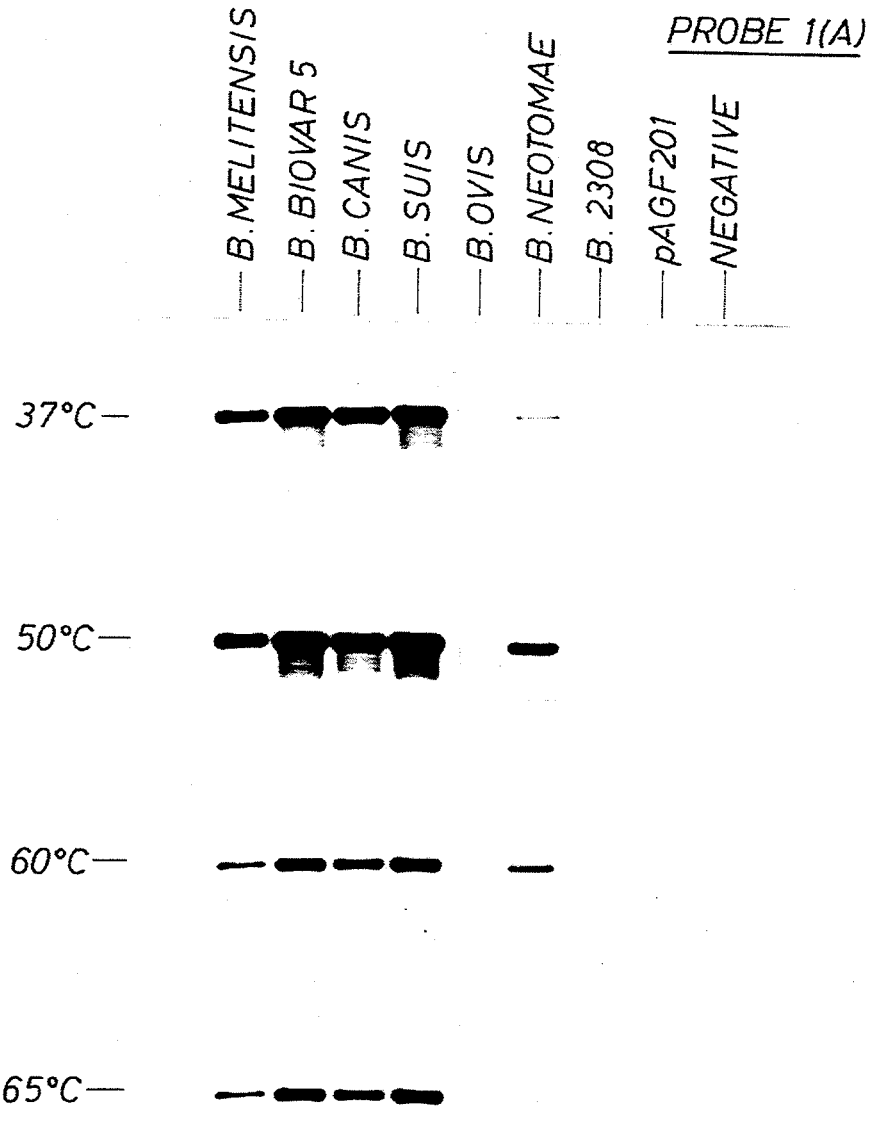

PROBE 1(A)
B. MELITENSIS
B. BIOVAR 5
B. CANIS
B. SUIS
B. OVIS
B. NEOTOMAE
B. 2308
pAGF201
NEGATIVE
37°C
50°C
60°C
65°C

FIG. 9

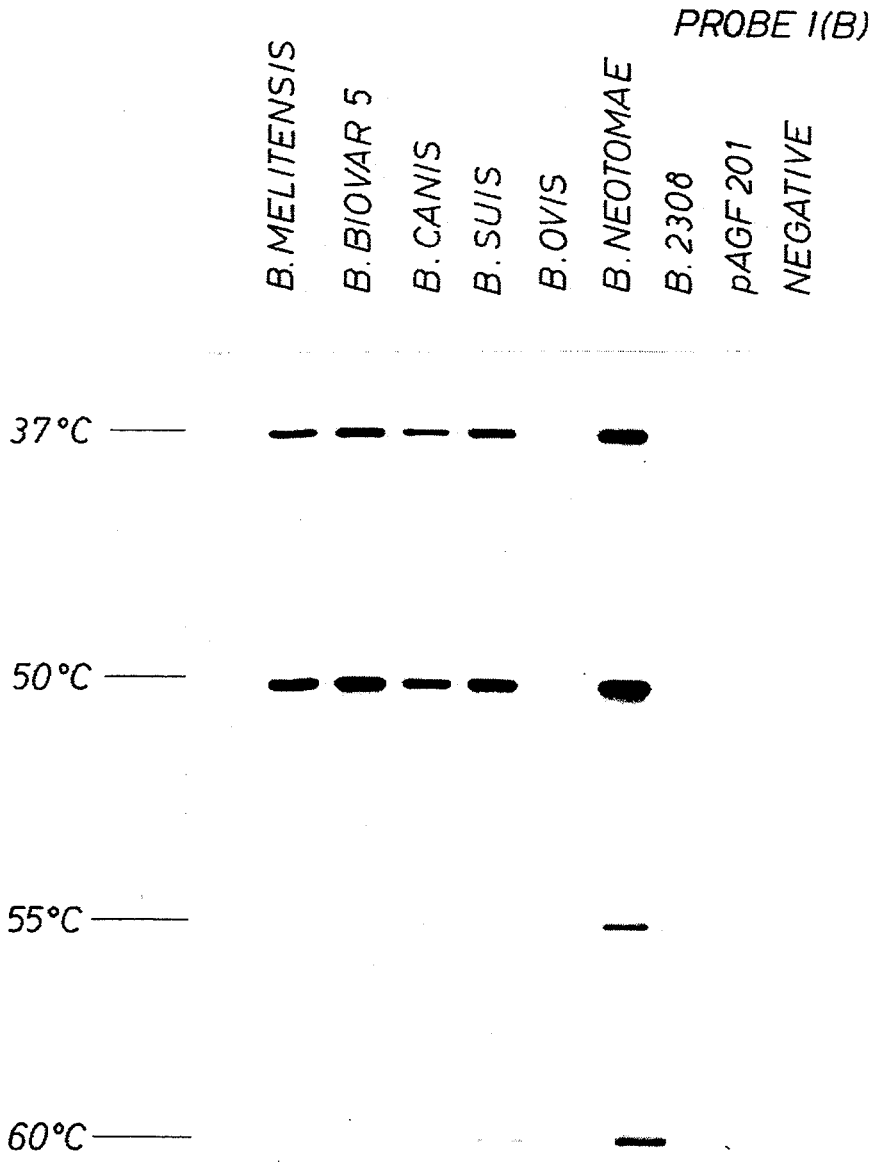

PROBE I(B)
B. MELITENSIS
B. BIOVAR 5
B. CANIS
B. SUIS
B. OVIS
B. NEOTOMAE
B. 2308
pAGF 201
NEGATIVE
37°C
50°C
55°C
60°C

FIG. 10

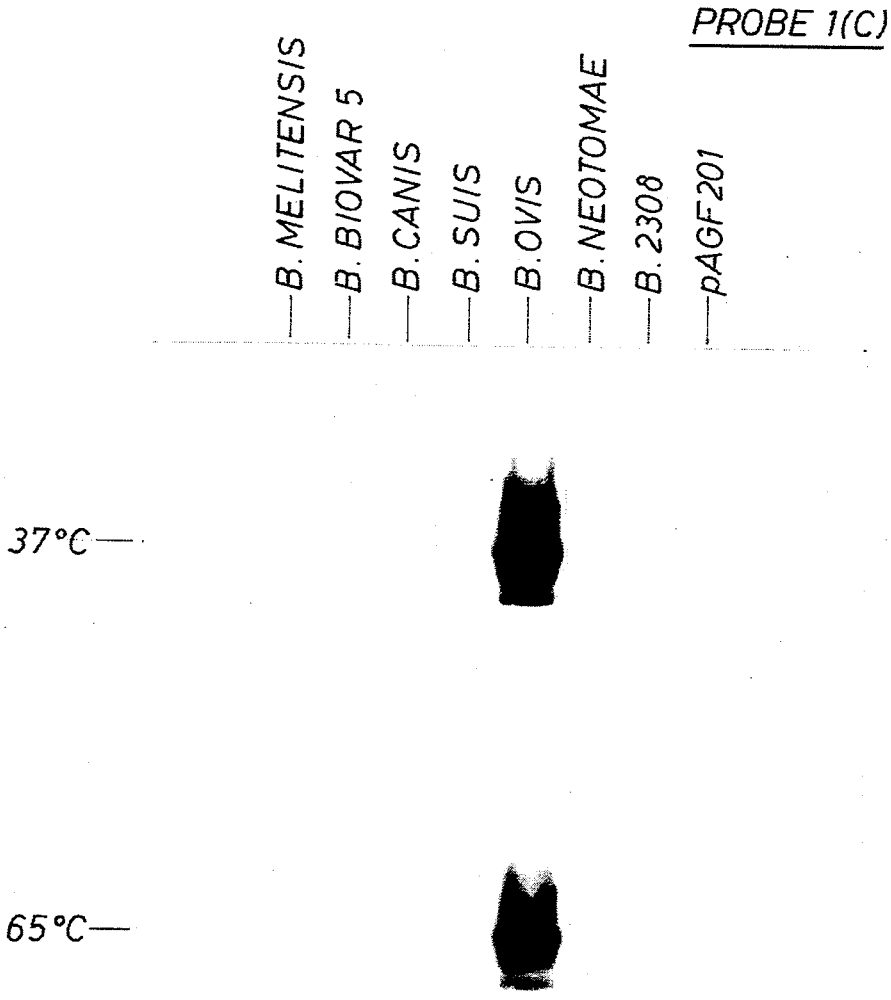

PROBE 1(C)
B. MELITENSIS
B. BIOVAR 5
B. CANIS
B. SUIS
B. OVIS
B. NEOTOMAE
B. 2308
pAGF201
37°C
65°C

FIG.11

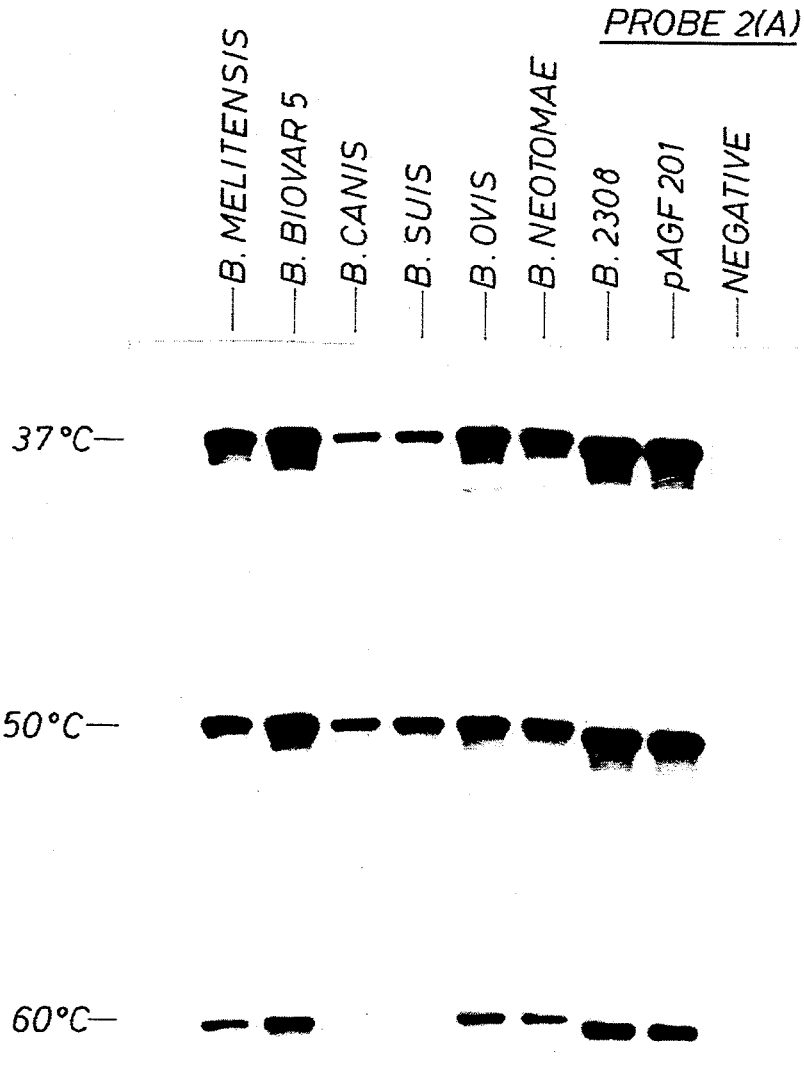
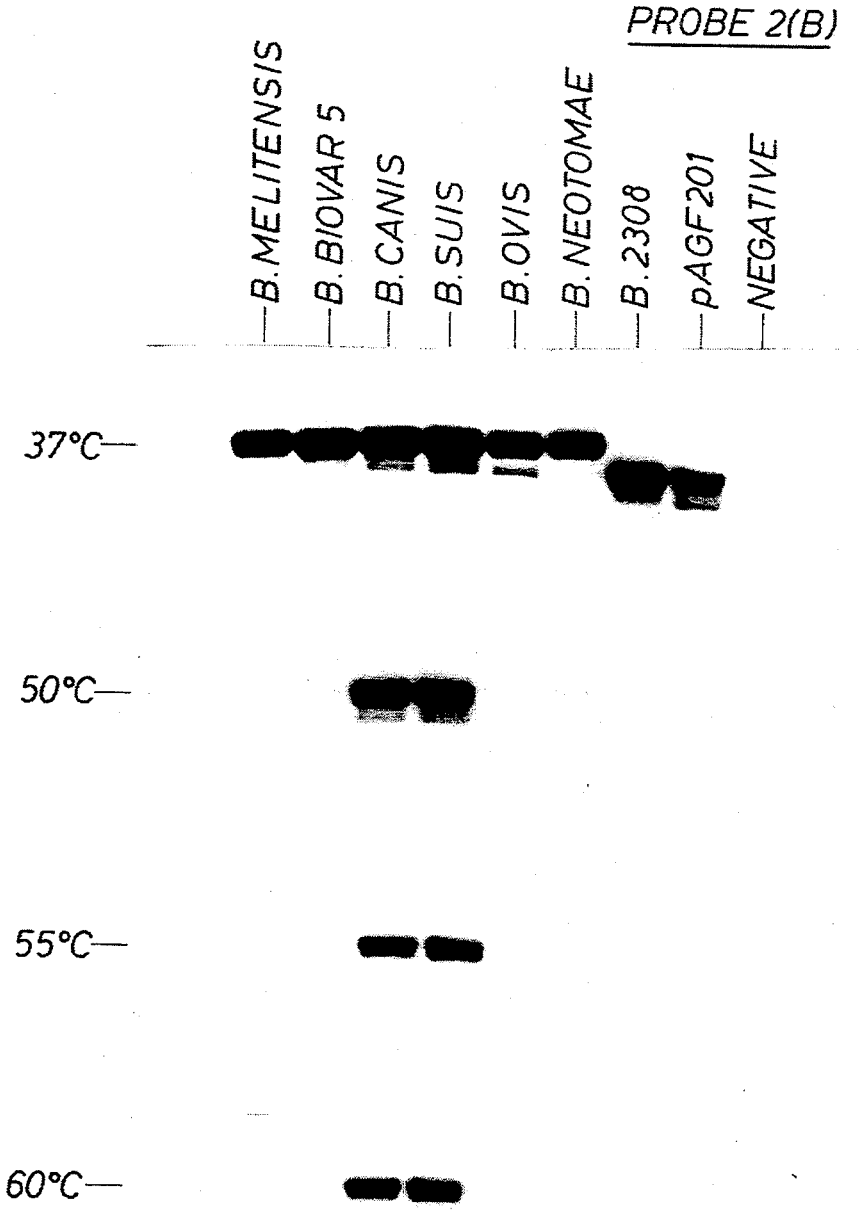

PROBE 2(A)
B. MELITENSIS
B. BIOVAR 5
B. CANIS
B. SUIS
B. OVIS
B. NEOTOMAE
B. 2308
pAGF 201
NEGATIVE
37°C
50°C
60°C

FIG. 12


PROBE 2(B)
B. MELITENSIS
B. BIOVAR 5
B. CANIS
B. SUIS
B. OVIS
B. NEOTOMAE
B. 2308
pAGF201
NEGATIVE
37°C
50°C
55°C
60°C

PROBES AND METHOD FOR IDENTIFYING SPECIES AND BIOVARS OF BRUCELLA

This is a continuation-in-part of U.S. Ser. No. 07/527,017 filed May 22, 1990.

FIELD OF THE INVENTION

This invention relates to a method for the diagnostic identification of the pathogenic bacterium Brucella, and more specifically to novel oligonucleotide probes and methods to identify a species and biovar of Brucella.

BACKGROUND OF THE INVENTION

Brucella is a genus of pathogenic bacteria which cause acute or chronic illness in many animal species, including humans and cattle. Six species of and multiple biovars have been characterized by phenotypic methods, although such methods are not always reliable. The six species and multiple biovars of Brucella may also be characterized by their natural host and a strain's geographical origin (See Table 1), however, a species may infect an animal other than fits natural host, and a single strain may now be found fin multiple geographic locations.

Early detection and characterization of the species or biovar of the infecting Brucella organism would be of great value in medical and veterinary practice. Rapid and reliable detection of Brucella infection is important to permit removal of infected animals from a healthy herd and prevent the spread of the disease. Characterization of the species or biovar of Brucella would provide epidemiological data to determine the source of the infection.

TABLE 1

SPECIES	BIOVAR	STRAIN	HOST	ORIGIN
B. abortus	1	19		U.S.
	1	2308	cattle	U.S.
	1	RB51	d.2308[a]	U.S.
	1	45/20	d.45/0	England
	2	ATCC 23449	cattle/ bison	England
	3	ATCC 23450	cattle/ bison	Uganda
	4	ATCC 23451	cattle/ bison	England
	5	ATCC 23452	cattle/ bison	England
	6	ATCC 23453	cattle/ bison	Africa
	7	ATCC 23454	cattle/ bison	
	9	ATCC 23455	cattle/ bison	England
B. melitensis	1	ATCC 23456	goat	U.S.
B. suis	1	ATCC 23444	pig	U.S.
B. neotomae		ATCC 23459	desert wood rat	U.S.
B. canis		ATCC 23365	dog	U.S.
B. ovis		ATCC 25840	sheep	Africa

ATCC - American Type Culture Collection, Bethesda, Maryland.
d. - derivative

Heretofore, standard serological tests used to detect Brucella have required several weeks time to complete and have not always been able to distinguish between species of Brucella. The methods currently available to identify species of infecting Brucella require the isolation of bacteria on selective media followed by quantitative analysis of phenotypic properties of the organism. Phenotypic characterization may be based on such features as lipopolysaccharide antigens, phage typing, dye sensitivities, CO_2 requirements, H_2S production, and metabolic properties. Such methods are time consuming (requiring 1–4 weeks) and are unreliable. (see Alton, 1988, *Techniques for the Brucellosis Laboratory*; Moriera-Jacob, 1963, *Nature* 197: 406; Shibata, 1962, *Nat. Inst. Anim. Health Q.* 2: 10–14) Time delays in obtaining test results and uncertainty due to unreliable test results can result in great economic losses. Suspect animals may require quarantine or may contaminate healthy animals in the herd during the waiting period.

Identification of Brucella species using DNA probes has not previously been possible, due to the high degree of inter-species DNA homology (approximately 90%).

There remains a great need for a rapid and accurate method for detecting the presence of pathogenic Brucella organisms in a suspect animal. It is also greatly desirable that such a detection method have the ability to distinguish between and identify the species and/or biovars of Brucella.

SUMMARY OF THE INVENTION

The method of the present invention solves the problems of the prior art methods by providing a rapid, sensitive, and accurate diagnostic method for the detection of Brucella and, more specifically a diagnostic method which is able to distinguish between species and biovars of Brucella.

It has now been found that the omp2 gene locus is conserved in all species of Brucella. Rapid detection of Brucella is achieved by identification of the conserved omp2 gene locus.

It has also been found that genetic variation at the omp2 gene locus of Brucella correlates with established species designations, and that this genetic variation may be used as a stable diagnostic marker for particular species of Brucella. Differentiation between species and biovars is based upon analysis of restriction fragment length polymorphism in the omp2 gene locus of Brucella.

A preferred embodiment of the method of the present invention includes amplification of the omp2 gene locus. The amplified omp2 gene locus may then be analyzed directly by electrophoretic separation, dot blot or Southern Blot analysis to enable diagnosis of Brucella infection. Alternatively, restriction digestion of the amplified omp2 gene releases fragments which may be analyzed, for example by gel electrophoresis, and the restriction fragment pattern used to detect the presence of Brucella and to identify the species or biovar of Brucella.

In the preferred embodiment, novel DNA probes which hybridize to the omp2 gene locus are used to enable diagnosis of Brucella infection, and additional DNA probes having specific hybridization characteristics are used to identify the species or biovar of Brucella.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a partial restriction map of the omp2 locus of the different Brucella species and biovar groups.

FIGS. 2A–O are an aligned sequence listing of the omp2 gene locus of different species and biovars of Brucella, with underlined sequences depicting oligonucleotides useful as diagnostic probes.

FIG. 3 is a partial restriction map of the omp2 locus of *Brucella abortus.*

FIG. **4** is a diagram of the Brucella omp2 gene locus showing locations of amplification primers useful in the present invention.

FIG. **5** is a Southern Blot of Brucella genomic DNA digested with Bam HI and hybridized with a labeled Bam HI fragment containing the omp2 gene locus of *B. abortus.*

FIG. **6** is a Southern Blot of Brucella genomic DNA digested with Pst I and hybridized with a labeled Bam HI fragment containing the omp2 gene locus of *B. abortus.*

FIG. **7** is an agarose gel stained with ethidium bromide showing the presence of the amplified omp2 gene in Brucella infected versus non-infected cattle.

FIG. **8** is a Southern Blot of amplified Brucella omp2 DNA hybridized with the diagnostic probe 1a.

FIG. **9** is a Southern Blot of amplified Brucella omp2 DNA hybridized with the diagnostic probe 1b.

FIG. **10** is a Southern Blot of amplified Brucella omp2 DNA hybridized with the diagnostic probe 1c.

FIG. **11** is a Southern Blot of amplified Brucella omp2 DNA hybridized with the diagnostic probe 2a.

FIG. **12** is a Southern Blot of amplified Brucella omp2 DNA hybridized with the diagnostic probe 2b.

DETAILED DESCRIPTION OF THE INVENTION

According to the method of the present invention, animal fluids or tissues may be tested for the presence of Brucella, and the species and biovar of Brucella infecting the animal may be rapidly and accurately detected. Animal fluids and tissues including blood, urine, milk, semen, vaginal secretions, rectal secretions or other available tissues may be collected and used as the test sample, despite the presence of complex, non-Brucella DNA. The live bacteria in the sample are killed, for example by heating to 68° C. for approximately 1 to 2 hours. The cells of the sample are then lysed to release DNA, for example, by heating to approximately 95° C. for approximately ten minutes or by repeated freezing and thawing of the cells. It may be desirable to immobilize the released DNA on a solid support in order to concentrate the DNA. For example, the DNA released by the lysed cells may be collected and concentrated in an agarose gel, or on a nitrocellulose filter.

A desired gene sequence in the DNA released from the lysed cells is then amplified, preferably through 30 to 50 cycles, by means of standard liquid polymerase chain reaction (PCR) using commercially available cyclers or manually in changing water baths. The PCR method is known in the art, and is described, for example, in Saiki et al, *Science* 239: 487–491, 1985, which is hereby incorporated by reference. In general, the PCR amplification method includes the hybridization of a pair of oligonucleotide primers to a segment of DNA. The oligonucleotide primers are designed to anneal to the DNA sequences flanking the target gene sequence that is to be amplified, with one oligonucleotide upstream and one downstream of the target sequence, on opposing DNA strands. During each amplification cycle, DNA strands are separated, for example by heating, priming oligonucleotides are annealed, for example by cooling the heated DNA in the presence of the oligonucleotides, and the primers are extended using DNA polymerases and adding nucleotides to the end of each primer to make copies of the target DNA sequence. This process is repeated through approximately 30–50 amplification cycles, geometrically increasing the number of copies of the target gene sequence.

Specific oligonucleotides are used to prime the amplification at the omp2 gene locus. As shown in FIG. **1**, the omp2 gene locus includes the omp2a and omp2b genes as well as flanking and intervening gene sequences. The DNA sequences of the omp2 gene locus of various Brucella species and biovars is shown in FIGS. **2A–O** and listed as Sequence Id. Nos. 2–8. A consensus sequence (Seq. Id. No. 1) is also shown in FIGS. **2A–O**.

Specific oligonucleotide pairs designed to hybridize to specific gene sequences of the omp2 gene locus permit amplification of a desired gene sequence of the omp2 gene locus. In the method of the present invention, regions of the omp2 gene locus having sufficient diversity to enable identification of Brucella species and biovars are amplified. Examples of oligonucleotides useful in the present invention include the amplification primers listed in Table 2. In FIGS. **2A–O**, those oligonucleotides useful as amplification primers are overlined in the consensus sequence. Useful primers are also shown in FIG. **4**. Preferred are primers which amplify the Brucella omp2 gene locus in the region approximately between base pairs 2470 and 3360 of the consensus sequence, due to o the unusually high density of species variation in this region. The primer set having Seq. Id. Nos. 19 and 20 is particularly useful to amplify this region. While it is understood that several regions of sequence diversity found in the omp2 gene locus may potentially be used to prepare diagnostic probes, the preferred DNA sequences amplified in the present invention include regions of sufficient DNA homology among Brucella species to enable PCR amplification and confirmation of identity as well as sufficient sequence diversity to permit the characterization of the specific species or biovars of Brucella.

TABLE 2

OLIGONUCLEOTIDE PAIRS TO AMPLIFY BRUCELLA OMP2 GENE

PROBE NO.	SEQUENCE ID. NO.	SEQUENCE	AMPLIFIED
47		CGC GAA CTC CAT GAC GGT GCC GC	omp2b
41		CCT TGG CTC CGC TGC AGC TCT GGT	
32	11	CAG GCG ATC TTC CGC GAC CCC	omp2b
33	12	GGG GAT GGG GAC AGG TTG TCC	
51	13	TGG GTC TGG GCA TTC TGA TTT GGC TG	intervening
50	14	TCG CCA GAA TTT TGA ATA GCC ATT AC	
41	15	CCT TGG CTC CGC TGC AGC TCT GGT	omp2a
46	16	CGT TGT CAA CGT CTT CGC CAC CC	
34	17	CCG GCG GCC AAC GGG AAA CCG	omp2a
35	18	CGG CTT TAC CCC TCG CGC AC	
20	19	TGG CTC AAT CCT TTA CAA	omp2a
21	20	TCG TGA TGT CGC TGA TGG	

The amplified DNA may be analyzed directly by dot blot analysis using a labeled omp2 gene probe, by hybridization analysis using radiolabeled oligonucleotide probes, or by separating the amplified DNA, for example, using agarose gel electrophoresis and ethidium bromide staining or Southern Blot analysis to detect the amplified gene sequence. The presence of the amplified omp2 gene indicates the presence of Brucella organisms in the test sample.

In a preferred embodiment, the amplified DNA may first be digested with specific restriction enzymes to generate restriction fragments characteristic of the omp2 gene locus prior to analysis by separation and staining or hybridization to specific omp2 gene probes. Proper selection of the restriction enzyme may result in fragments displaying an electrophoretic pattern characteristic of the Brucella omp2 gene in all species of Brucella. Alternatively, the selection of restriction enzymes may result in fragments displaying restriction fragment length polymorphism (RFLP), for example, in the omp2a gene and flanking sequence of Brucella.

A preferred restriction enzyme which can be used to detect the omp2 gene in all species of Brucella is Bam H1. Restriction digestion of genomic or amplified Brucella DNA using Bam HI releases a characteristic 6.5 kb fragment containing the omp2 gene.

Preferred restriction enzymes which can be used to identify the particular species or biovar of the infecting Brucella organism include PstI and KpnI. Digestion of the amplified omp2 gene locus with PstI and/or KpnI results in restriction fragments displaying a unique electrophoretic pattern in agarose gels for *B. abortus, B. melitensis, B. canis,* and *B. ovis.* The restriction fragment patterns for *B. suis* and *B. neotomae,* while distinct from the other 4 Brucella species, are not distinguished from each other using these digestive enzymes. Biovars 1, 2, and 4 of *B. abortus* may also be identified based upon the size of the PstI restriction fragments.

The pattern of restriction fragments may be visualized in the electrophoretic gel by staining, for example, with ethidium bromide, which has a sensitivity in the range of 0.1–1.0 μg DNA. Alternatively, when the amount of DNA is limited, i.e., 0.01–0.1 μg DNA, Southern Blot or dot blot analysis with omp2 DNA probes can be used.

In a preferred embodiment, novel DNA probes are used to identify a Brucella species or biovar. These probes can be used in hybridization analysis such as dot blot or Southern blot following radioactive labeling or other method of detection, e.g., chemiluminescence or color-development. For example, oligonucleotide probes in the region amplified by the amplification primers having Seq. Id. Nos. 19 and 20 can be used to specifically diagnose a Brucella species or biovar and are shown in Table 3.

TABLE 3

SEQ. ID.	PROBE		
21	1a	ATGTCGTCGC	TGCTGGCTCC
22	1b	ATGTCGTCGC	TGATGGCTCC
23	1c	ACGTGATCTC	GGCTGGCTCC
24	2a	TGTTGTTGCC	TATGACTCGG
25	2b	TGCTGTTGCC	TATGACCCGG
26	3a	CCCCGAAAAG	GCAACCTTCA
27	3b	CACCGAAAAG	GCAACCTTCA
28	3c	CACCGAAAAG	GTAACCTTCA
29	4a	AGACCGCAGT	TACCGCCAAC
30	4b	AGACGGCAGT	TACGGCTAAC
31	5a	GTCGCTTATC	AGCTCGTTCC
32	5b	GTTGCTTACG	AACTGGTTCC

In the method invention, oligonucleotide primers are used to amplify a specific region of the omp2 gene which contains sufficient diversity in DNA sequence among Brucella species and biovars to permit their identification. Oligoneucleotide probes which hybridize to sequences contained within the amplified DNA region are used to specifically identify the species and/or biovar of Brucella. It is understood that regions of species diversity in the omp2 gene locus may be used to distinguish between species and biovars of Brucella. It is preferred that the region of the omp2 gene to be used contain sufficient diversity among species and biovars of Brucella to enable identification using a minimal number of diagnostic probes.

Primers such as those having Seq. Id. Nos. 19 and 20 are used to amplify a region of the omp2 gene having sufficient diversity to enable distinction of species and biovars of Brucella. As shown in FIGS. 2A–O, DNA amplified between Seq. Id. Nos. 19 and 20 correspond to areas of the Brucella omp2 gene consensus sequence at approximately nucleotides 3358–3376 and 2444–2461, respectively. These primers amplify a region of Brucella omp2 DNA having a very high degree of diversity among species and biovars of Brucella, as shown in FIGS. 2A–O. The probes listed in Table 3 hybridize to DNA of specific species or biovars of Brucella in the region between these primers, in a pattern which permits specific identification.

TABLE 4

	1			2		3			4		5	
	a	b	c	a	b	a	b	c	a	b	a	b
B abortus b1	−	−	−	+	−	+	−	−	+	−	+	−
B abortus b5	+	−	−	+	−	+	−	−	+	−	+	−
B. melitensis	+	−	−	+	−	−	+	−	+	−	+	−
B. suis	+	−	−	−	+	−	−	+	+	−	+	−
B. canis	+	−	−	−	+	−	+	−	+	−	+	−
B. neotomae	−	+	−	+	−	−	+	−	−	+	−	+
B. ovis	−	−	+	+	−	−	+	−	+	−	+	−

A diagnostic test to distinguish among the Brucella species can be performed using a combination of these oligonucleotide probes. The basic premise is to characterize the amplification products of a PCR reaction by hybridization. Reference to Table 4 indicates use of a single probe, for example, probe 5b, may be sufficient to diagnose *B. neotomae.* Likewise, probe 3c can diagnose *B. suis.* Generally, however, the diagnosis cannot be performed with a single oligonucleotide due to the similarity in the DNA of these organisms, and thus a combination of at least two probe sets is used to distinguish Brucella species and biovars. Table 4 shows the pattern of hybridization for each of the probes listed in Table 3 with omp2 DNA of specific species and biovars of Brucella. Comparison of hybridization results of a test sample to a panel of two or more probes known to hybridize in a specific pattern with the omp2 gene of specific species and biovars of Brucella, for example, those shown in Table 4, enables identification of a specific species or biovar of Brucella in a test sample.

For example, as shown in Table 5, characterization of the amplification product of the omp2a gene via hybridization to oligonucleotide probes 1(a–c) and 3(a–c) dis-

tinguishes all Brucella species except *B. melitensis* and *B. canis*. An additional probe set, 2(a-b), distinguishes *B. melitenis* and *B. canis*. In a similar fashion, other probes identified in FIGS. 2A–O may be used in various combinations to provide a diagnostic panel of oligonucleotides to identify specific Brucella species and biovars.

TABLE 5

	POSITIVE HYBRIDIZATION		
	1	2	3
B. abortus b1	—	a	a
B. abortus b5	a	a	a
B. melintensis	a	a	b
B. suis	a	b	c
B. canis	a	b	b
B. neotomae	b	a	b
B. ovis	c	a	b

Using the omp2 gene sequences disclosed in FIG. 2, additional regions of the omp2 gene locus may be identified for amplification or additional oligonucleotide probes may be identified for use in diagnosing Brucella infection in a manner similar to that described herein. It is also understood that the oligonucleotides disclosed herein may be modified, e.g., by shifting the sequence upstream or downstream, to attain similar amplification and probing results as described for the exemplified oligonucleotides.

The invention may be better understood by reference to the following examples.

EXAMPLES

EXAMPLE 1

Conservation of the omp2 Gene Locus in Species and Biovars of Brucella

B. abortus smooth strains 19 and 2308 were obtained from Dr. Billy Deyoe at the National Animal Disease Center in Ames, Iowa. *B. abortus* biovars 1–7 and 9, *B. suis, B. canis, B. neotomae, B. melitensis*, and *B. ovis* were obtained for the American Type Culture Collection, in Bethesda, Md. (See Table 1). Strain identification was confirmed by standard biovar analysis (see Alton, 1988). Brucella strains were cultivated on either Brucella agar or tryptic soy agar (Difco Laboratories, Detroit, Mich.). *E. Coli* cells were grown as described in Ficht, 1988, *Infect. Immunol.* 56: 2036–2046.

Brucella cells were grown on agar plates at 37° C. for approximately 48 hours. Cells were washed off the plates in 5 ml of phenol/saline (0.1% w/v and 0.85% w/v, respectively). The cells were killed by incubation for 1–2 hours at 68° C. and pelleted by centrifugation at 5000 rpm for 20 minutes. The cell pellet was resuspended in 5 ml buffer A (10 mM Tris-HCl, pH 7.6, 1M NaCl) at room temperature, pelleted again, and resuspended in a final volume of 2 ml buffer A. The cell suspension was warmed to 42° C. and diluted with an equal volume of a solution containing 1% w/v low melting point agarose (Bethesda Research Labs, -Bethesda, Md.) in sterile water. Aliquots (100–200 μl) of this mixture were poured into molds to form agarose blocks and chilled on ice. The blocks were transferred to Eppendorf tubes containing an equal volume of lysis buffer (6 mM Tris-HCl, pH 7.6, 1M NaCl, 100 mM EDTA, pH 7.5, 0.5% w/v Brij-58 (Aldrich, Milwaukee, Wis.), 0.2% w/v sodium deoxycholate, 0.5% w/v sodium N-lauroylsarcosine) made from sterile stock solutions and filter sterilized following the addition of detergents. This solution was supplemented just prior to use with 1 mg/ml lysozyme and 20 μg/ml RNase A (10 mg/ml stock in sterile dH_2O heated to 80° C. for 20 minutes). The cell suspension was then incubated in the lysis buffer overnight at 37° C. The following day the lysis buffer was removed and an equal volume of ESP buffer (0.5M EDTA, pH 9–9.5, 1% w/v in sodium lauryl sarcosinate, and 1.0 mg/ml proteinase K preincubated for 2 hours at 37° C.) was added. The mixture was incubated for 24–48 hours at 50° C. The gel block was then washed in 4 changes of TE buffer (50 mM Tris-HCl, 0.1 mM EDTA, pH 7.5) containing 1 mM phenyl methyl-sulfonyl fluoride (PMSF) for 4 hours at room temperature. The gel block was then washed twice for 4–16 hours with Bam HI restriction enzyme buffer (as supplied by the manufacturer, Boehringer-Mannheim, Indianapolis, Ind.) The washed block was dissolved in 0.5 ml of the restriction enzyme buffer at 65° C. for 10 minutes.

The restriction fragments were separated in a 2% w/v agarose gel. Southern Blot analysis included the transfer of the separated restriction fragments onto nitrocellulose, and hybridization with a labeled DNA probe consisting of the Bam HI restriction fragment of the *B. abortus* omp2 gene locus, as shown in FIG. 3. The results of the Southern Blot analysis are shown in FIG. 5, and indicate that all six species of Brucella and all *B. abortus* biovars tested have conserved the omp2 locus on a 6.5 kb Bam HI fragment.

EXAMPLE 2

Heterogeneity of the omp2a Gene in Species and Biovars of Brucella

Aliquots of Brucella DNA prepared for Example 1 were treated as described in Example 1, but digested with Pst I in Pst I restriction enzyme buffer (as provided by the manufacturer, Boeringer- Mannheim). Electrophoresis and Southern blot analysis were carried out as described for Example 1. The results of the Southern Blot analysis are shown in FIG. 6, and indicate that the genetic variation of the omp2 locus segregated along classical species lines, that is the Pst I restriction fragment profiles of the omp2 gene locus were distinct for different species and biovars of Brucella. Based on Pst I restriction digestion, the species can be divided into six groups as shown in FIG. 1. Group 1 includes *B. abortus* biovars 1, 2 and 4. Group 2 includes *B. abortus* biovars 3, 5, 6, 7 and 9. Group 3 includes only *B. melitensis*. Group 4 includes *B. suis* and *B. neotomae*. Additional restriction digestion with the restriction enzyme Kpn I enabled distinction of Group 5, *B. canis* from the species of Group 4. Group 6 contains only *B. ovis*.

This data indicates that after one restriction digest with Pst I, analysis of the restriction fragments can distinguish between *B. abortus, B. ovis, B. melitensis*, and the remaining species of Brucella. Restriction fragments generated from Pst1 digestion can also distinguish between *B. abortus* biovars 1, 2 and 4 from *B. abortus* biovars 3, 5, 6, 7 and 9. Additional digestion with Kpn I permits the distinctive identification of *B. canis*.

EXAMPLE 3

Detection of Brucella in Tissue Samples of Infected and Control Cattle by Amplification of omp2 DNA

Cattle (two) (mixed breed, (*Bos Taurus*×*Bos Indicus*, Montana Beaver Head Ranch, Big Hole, Mont.), at approximately 120 days gestation, were infected with

1×10^7 *B. abortus* S2308 organisms (obtained from Dr. Billy Deyoe, U.S.D.A. N.A.D.C.). Abomasal tissue samples were prepared from either aborted calves or live calves of the two infected animals and two noninfected control animals.

Abomasal tissue samples were obtained by necroscopy following animal sacrifice. The fetal stomach or abomasum and its contents were dissected and stored in whirlpak bags (NASCO, Fort Atkinson, Wis.). Portions of the abomasal samples were heated at 68° C. for 2 hours in eppendorf tubes to kill any live Brucella, and 5 μl portions were then added to amplification reactions according to the method of Saiki et al, 1985.

The standard amplification reaction was performed in a final volume of 100 μl containing 200 μM of each nucleotide (dNTP, dGTP, dCTP, dTTP), 2.5 units Taq polymerase, approximately 0.1 ng template DNA and 1 μM each oligonucleotide primer. The reaction buffer also contained 10 mM TRIS-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01% w/v gelatin. The oligonucleotides used to prime the amplification were No. 32 and No. 33, as shown in Table 2, which amplified the omp2b gene of Brucella.

The DNA was amplified through 30 cycles including a 30 second melting at 94° C., annealing over 30 seconds at 62°–65° C., and polymerization at 72° C. for three minutes.

The amplified DNA was then sized in a 1–2% w/v agarose gel and stained with ethidium bromide to visualize the DNA. As shown in FIG. 7, DNA obtained from infected animals amplified the 1268 bp omp2b gene fragment characteristic of Brucella. Non-infected animals showed no amplified DNA product.

EXAMPLE 4

Detection of Brucella in Milk Samples From Infected and Control Cattle

Pregnant cattle (six) (*Bos Taurus*×*Bos Indicus*, Montana Beaver Head Ranch, Big Hole, Mont.), at approximately 120 days gestation were infected with 1×10^7 *B. abortus* S2308 (obtained from Dr. Billy Deyoe) organisms by conjunctival installation. Four uninfected cattle served as controls. The animals are monitored serologically for infection until abortion or the birth of a live calf. Samples collected for bacteriologic analysis are used for PCR amplification and DNA analysis of Brucella.

Blood samples are collected weekly; sera is tested by the following methods:

1. buffered Brucella antigen (Card) (O'Reilly and Cunningham, 1971, *Vet. Rec.* 88: 590–594; Ladwig, 1968, *Iowa Vet.* 39: 9–14).
2. enzyme linked immunosorbent assay (ELIZA) (Heck et al, 1980, *Am. J. Vet. Res.* 41: 2082–2084).
3. rivanol precipitation plate agglutination (Huber and Nicoletti, 1986, *Am. J. Vet. Res.* 47: 1529–1531).
4. automated tube (warm) complement fixation with Brucella antigens and hemolysis in gel test (Timbs et al, 1978, *N.Z. Vet. J.* 26: 52–56; Nicoletti and Carlsen, 1981, *Am. J. Vet. Res.* 42: 1494–1497).

Vaginal and rectal swabs, placental and quarter milk samples from all parturient cattle will be cultured for Brucella. Rectal swabs from viable calves, and pulmonary tissue, gastric contents, mediastinal lymph nodes, and rectal swabs from dead fetuses or neonates will be streaked onto semi-restrictive Brucella agar medium with 5% bovine serum and antibiotics (Farrell's Medium, Farrell et al, 1974, *Res. Vet. Sci.* 16: 280–286).

Culture negative parturient principals and controls will be euthanized and at least 50 tissues will be collected, trimmed of non-lymphatic tissue, and both sides of the cut surface will be rubbed over the surfaces of Farrell's media (3 plates per tissue sample). Inoculated media will be incubated at least 7 days at 37° C. in 10% CO_2 with bacterial colonies resembling Brucella further identified and biotyped by conventional methods for comparison with results for the same animal from PCR amplification and analysis of DNA from tissue and fluid samples.

DNA obtained from blood, milk, semen, vaginal secretions, rectal secretions and tissue samples will be concentrated if necessary onto nitrocellulose filters. The DNA obtained will be amplified according to the procedure described for Example 3, using oligonucleotides which amplify specific regions of the omp2 gene locus. Amplification of the omp2b gene locus and identification of the omp2b gene as described for Example 3 will identify the presence of Brucella organisms. Amplification of the omp2a gene using the oligonucleotides No. 34 and No. 35, as shown in Table 2, followed by electrophoretic analysis of the amplified sequence will be used to determine the presence of Brucella in the test sample. Restriction digestion of the amplified DNA sequence using the enzyme Pst I will characterize the infecting Brucella species as *B. abortus* biovars 1, 2, 4, *B. abortus* biovars 3, 5, 6, 9. *B. ovis, B. melitensis*, or one of the remaining three Brucella species. Restriction digestion using the enzyme Kpn I will distinguish *B. canis* from the remaining two Brucella species, *B. suis* and *B. neotomae*.

EXAMPLE 5

Identification of Brucella Species and Biovars Using Specific Hydridization Probes

DNA was extracted by from each of the known Brucella species and from *B. abortus* biovars 1 and 5. A diagnostic region of the omp2 gene locus was PCR amplified using amplification primers having Seq. ID Nos. 19 and 20. Cells were added to a standard PCR reaction mix containing 1 X reaction buffer (10 mM Tris-HCl, pH 8.3/50 mM KCl), 1.5 mM $MgCl_2$ 200 μM of each dNTP, 0.2 μM of each primer and 1 unit of Taq or Tth polymerases. Amplification primers (Seq. Id Nos. 19 and 20) were added to initiate the reactions during a hot start preincubation step at 84° C. This was followed by an initial 94° C. heating prior to thermal cycling. Standard reaction conditions can also be employed using 94° C. melting, 50° C. annealing, and 72° C. polymerization. The products of these reactions were electrophoresed on a 1.5% agarose gels and transferred to nylon membranes. The nylon filter was prehybridized overnight at 68° C. for 16–24 hours in 50 ml prehybridization buffer (6XSSPE, 0.1% (w/v) NaPPi, 0.1% (w/v) SDS, 0.5% (w/v) blotto, 0.1 mg/ml ssDNA, and 0.02 mg/ml *E. coli* tRNA).

Oligonucleotides were labeled by mixing the following:

α[^{32}P]-ATP (6000 Ci/mmol)	100 μCi
oligonucleotide (20 μg/ml)	1 μl
5X Tailing buffer (Boehringer Mannheim)	3 μl
$CoCl_2$	4.5 μl
TdT (55 units/μl)	1 μl

-continued

dH_2O	to 15 μl

The mixture was incubated at 37° C. for 30 minutes. The labeled oligonucleotides (25/200 μl) were passed over a G-25 column and placed in 4 ml 6XSSPE containing 0.1% (w/v) SDS.

The filter containing the amplified DNA was hybridized in the labeled oligonucleotide solution overnight usually at 37° C. but no more than $T_m-5°$ C., which was calculated from the following formula: Tm=4(G+C)+2(A+T). After hybridization, the filter was washed 4 X at room temperature in 6XSSPE containing 0.1% (w/v) SDS, and at the appropriate temperature (depending on the desired stringency), blotted dry, wrapped in Saran wrap and exposed to X-ray film.

Amplified DNA from each Brucella species and biovar was hybridized with diagnostic oligonucleotide probes 1(a–c) and 2(a,b), at stringencies of 37, 50, 60, and 66° C. The resulting blots are shown in FIGS. **8–12**, and the pattern of specific probe hybridization is shown in Table 6.

TABLE 6

	1			2	
	a	b	c	a	b
B. biovar 5	+	−	−	+	−
B. melitensis	+	−	−	+	−
B. suis	+	−	−	−	+
B. canis	+	−	−	−	+
B. neotomae	−	+	−	+	−

Having described the invention above, various modifications of the techniques, procedures, materials, and equipment will be apparent to those in the art. It is intended that all such variations within the scope and spirit of the appended claims be embraced thereby.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 32

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3378 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CAGGCGATCT TCCGCGACCC CTGTAGAAAG ACTGCGGTCA GCATAAAAAG CAAGCATCTG      60
ATGCTGCACG AGGGCAACAA AAAACCCGGY ATTTCTGCCG GGTTTCTGTA TCCAATCCGT     120
AATGGATTAG AACGAACGCT GGAAGCGAAC GATACCGCCC CAAGCATTGT CTTCAGCAAC     180
RGTGTTCTTC CACTCGCCAC CAAACTTGGT GTAGGAAACT TCCGGMGYAA CGGTGAAGCC     240
AGGAACCAGT TCGTAAGCAA CGTTAGCCGT AACTGCCGTC TTGCCCCAGT CGTCATGCGC     300
AGCCTGCAGR TTGAAGGYWG CCTTYTSSGT RGCMWKRWAC TTYRSACCAC CCCAGACAGC     360
CCAATCGCCG CCCCACTGRC CGTAGTTCTG RTYCGGCGTM GCWGCGGACG AATATGCGCC     420
CTGCARCCAW ACCGAGAACY GGTCGGTGAT GTTGACGTCG CCACGAACCT TKGYAGCCCA     480
TTCTTCKATG ACCGAGTCAT AGGCAACAAC ACCAGCGATC GAACCCCAGC CGCCAGCATA     540
YTTCAGGCCG CCAACAACGT SMGGCATGTA RCCGTCGATS BKGTARTYGG TCGTGCCAGT     600
GTAAYYRYCR WCGTYKTCGC CACCCTGTTC GAGAGCGATC ACAGCCGAGA AGCCGTTTCC     660
GCCRGTGAAG GTGTASGMGA TCTTGCCGGT GCGGTAGGAG CCAGCCGAGA TCACGTCATC     720
GTTGATGAYA TCRCCGAGGT AACCGGTGAA GGTATGGAAT TCSGATTCRT CGATACCAAC     780
SYKSARACCA CCRAGCKKGA TATAYGCRAA CTSCAKRWCG GTGCCGSTGC TTACGCTGCC     840
ATCAGCGACA TCACGATCAT CGSTKWMATY RCCRTATTKR CCATCWWSRC SYGAATTGTT     900
SSYRGYRTAG TTGAAGCGCA GYKYRGTRWA GGTSYYGAGK GTGCCGAGTT CGGTTTCCGA     960
AYYSGTGTKR AACRYGGAGT GCRAAACGAG CRCYCTTGTC CCAGCCWTTR CGRTCSGWRC    1020
CGGWRTAAAC GTCRTCGCCG CCCTTTACGT CGTAACGGAC GTARCCRYKG AYGCGCAGGC    1080
AGGTTTCGGT GCCCGGAATG TAGAAGTAGC CAGCGCCRTA AGCGTCGCAA ACGCGGACAT    1140
```

-continued

```
ATTCAACGGC TTCGGGCTCT GGCGCGACGA TTGCGTCGGC AGCYTGAGCG CCGGAAGCTG   1200
CAACCAGAGC TGCAGCGGAG CCAAGGAGAA GGCTCTTGAT GTTCATTTCT GACCTCCAGT   1260
CAAAGTTAAA AATGGGTCTR GGCATTCTGA TTTGGCTGAA GGACAACCTG TCCCCATCCC   1320
CTAATTGAAA AAGTCGCCCC GAAGCGCTCC TTCTTCTGAA AGTGAAGATA CTCGCCCATT   1380
TATTCGTTTC AACATCGAAT ATGTTCTCAC AACCTTTAYG GTGCTGCTAT GAAGGGCAGT   1440
TRTTGCWGAA ATGACACRAA ATTACCTGCT TTAGCTCGGC GGATTCATGC TTTATTAACA   1500
TAAGTRAACG CGAATTAACC GATGTTAACG TTTGAAAATG CAAGTTTTTT AGGATCGCCT   1560
RCMGAATAAA GCCGCRRATC TTTCGTCGAA ACAGCCCTTA ACGGAATATG TCGGCAAGGT   1620
GGCAAGAATC GTCTGAACGG AGAGCAGAAA CCTCGAATCC GTTTCATTTA ATAAGGGCAA   1680
GTGCGTGCCG GTGCTAAATT GTGGGCCTTT TTAAGCGCGC YATATATATA AAGAGAATAA   1740
TCCGCAGGAA ATTTTACCAG TTAATGCGTA AATCGCTTGA AATGCCCAGG CGTACCGGTT   1800
ATCTCGCCTT TACCGGAGAG GTGGCCGAGT GGTCGAAGGC GCTCCCCTGC TAAGGGAGTA   1860
GACCTCAAAA GGGTCTCGTG GGTTCGAATC CCATCCTCTC CGCCAGTTTT TCCAATATCC   1920
CAGCAAATCT TTATGTGTTC GACGCGCTTG ATTTCATACG GAATCGGCTT TTACCCCTCG   1980
CGCACTGAAT CTCTGTTTTT CCAGGCTACG AATCCAGAAA ACAAGCAAGC CATTGATAAG   2040
TAATGGCTAT TCAAAATTCT GGCRATTCTT GACTGGAGGT CAGAAATGAA CATCAAGAGC   2100
CTTCTCCTTG GCTCCGCYGC AGCTCTGGTT GCAGCTTCCG GCGCTCARGC TGCCGACGCA   2160
ATCGTCGCGC CRGAGCCCGA AGCCGTTGAA TATGTCCGCG TTTGCGACGC TTAYGGCGCT   2220
GGCTACTTCT ACATTCCGGG CACCGAAACC TGCCTGCGCR TCMRYGGYTA CGTCCGTTAC   2280
GACGTAAAGG GCGGCGAYGA CGTTTAYWCC GGYWCSGAYC GYAAWGGCTG GGACAAGGGY   2340
GCTCGTTTYG CACTCATGTT CAACACGAAT TCGGAAACCG AACTCGGCAC ACTCGGCACC   2400
TATACTCAGC TGCGCTTCAA CTACACCAGC AACAATTCAC GTCATGATGG CCAATACGGC   2460
GATTTCAGCG ATGATCGTGA TGTCGCTGAT GGCRGCGTAA GCACCGGCAC CGATCTGCAG   2520
TTTGCATATA TCACGCTTGG TGGTTTCAAG GTTGGTATCG ACGAATCCGA ATTCCATACC   2580
TTCACCGGTT ACCTCGGTGA TRTCATCAAC GATGAYGTSR TCKCKGMTGG CTCCTACCGC   2640
ACCGGCAAGA TCGCCTACAC CTTCACCGGC GGAAACGGCT TCYCGGCTGT GATCGCTCTC   2700
GAACAGGGTG GCGAAGACGT TGACAACGAT TACACGATCG ACGGTTACAT GCCGCACGTT   2760
GTTGGCGGCC TGAAATATGC TGGCGGCTGG GGTTCGATCG CTGGTGYTGT TGCCTATGAC   2820
YCGGTCATCG AAGAATGGGC TACAAAGGTT CGTGGCGACG TCAACATCAC CGACCGGTTC   2880
TCGGTATGGC TGCAGGGCGC ATATTCGTCC GCAGCGACGC CGAACCAGAA CTACGGTCAG   2940
TGGGGCGGCG ATTGGGCTGT CTGGGGTGGT GCAAAGTTCA TTGCCMCCGA AAAGGYAACC   3000
TTCAATCTGC AGGCTGCGCA TGACGACTGG GGCAAGACSG CAGTTACSGC YAACGTYGCT   3060
TAYSARCTSG TTCCYGGMTT CACCRTTACG CCGGAAGTTT CCTACACCAA ATTTGGTGGC   3120
GAGTRGAAAG ACACCGTTGC TGAAGACAAT GCCTGGGGCG GTATCGTTCS YTTCCAGCGC   3180
TCGTTCTAAT CAGATCGACG TTAAGCATAG GGCGCCAACG GTTTCCCGTT GGCGCCGGTT   3240
CATTTGAAAC AGCGTTCACG AAAGCGTGAG AATCGATTCT TCCGGAATGG GGATTCCAGG   3300
CGGATCGACA ATTGAGGGAA TTGCGGGGAC GACAAAAAGC TGGGGGCAAC CGGGGGGTCT   3360
TGTAAAGGAT TGAGCCAM                                                 3378
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3347 base pairs
(B) TYPE: nucleic acid

-continued

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Brucella abortus
(B) STRAIN: biovar 5

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAGGCGATCT TCCGCGACCC CTGTAGAAAG ACTGCGGTCA GCATAAAAAG CAAGCATCTG 60

ATGCTGCACG AGGGCAACAA AAAACCCGGC ATTTCTGCCG GGTTTCTGTA TCCAATCCGT 120

AATGGATTAG AACGAACGCT GGAAGCGAAC GATACCGCCC CAAGCATTGT CTTCAGCAAC 180

GGTGTTCTTC CACTCGCCAC CAAACTTGGT GTAGGAAACT TCCGGCGTAA CGGTGAAGCC 240

AGGAACCAGT TCGTAAGCAA CGTTAGCCGT AACTGCCGTC TTGCCCCAGT CGTCATGCGC 300

AGCCTGCAGG TTGAAGGCAG CCTTCTGCGT AGCCTGATAC TTCAGACCAC CCCAGACAGC 360

CCAATCGCCG CCCCACTGGC CGTAGTTCTG ATCCGGCGTA GCAGCGGACG AATATGCGCC 420

CTGCAACCAA ACCGAGAACT GGTCGGTGAT GTTGACGTCG CCACGAACCT TGGCAGCCCA 480

TTCTTCGATG ACCGAGTCAT AGGCAACAAC ACCAGCGATC GAACCCCAGC CGCCAGCATA 540

CTTCAGGCCG CCAACAACGT CAGGCATGTA GCCGTCGATG TGGTAGTTGG TCGTGCCAGT 600

GTAACCACCG TCGTTGTCGC CACCCTGTTC GAGAGCGATC ACAGCCGAGA AGCCGTTTCC 660

GCCAGTGAAG GTGTACGAGA TCTTGCCGGT GCGGTAGGAG CCAGCCGAGA TCACGTCATC 720

GTTGATGACA TCGCCGAGGT AACCGGTGAA GGTATGGAAT TCCGATTCAT CGATACCAAC 780

GCGCAGACCA CCGAGCTGGA TATACGCGAA CTCCATGACG GTGCCGCTGC TGGTTTCATT 840

ACCATATTTA CCATCTACGC CCGAATTGTT CGCAGCATAG TTGAAGCGCA GTTCGGTGAA 900

GGTCTTGAGG GTGCCGAGTT CGGTTTCCGA ACCGGTGGAA ACGCGGAGTG CGAAACGAGC 960

GCTCTTGTCC CAGCCATTGC GGTCGGTACC GGAGTAAACG TCATCGCCGC CCTTTACGTC 1020

GTAACGGACG TAACCATGGA CGCGCAGGCA GGTTTCGGTG CCCGGAATGT AGAAGTAGCC 1080

AGCGCCGTAA GCGTCGCAAA CGCGGACATA TTCAACGGCT TCGGGCTCTG GCGCGACGAT 1140

TGCGTCGGCA GCCTGAGCGC CGGAAGCTGC AACCAGAGCT GCAGCGGAGC CAAGGAGAAG 1200

GCTCTTGATG TTCATTTCTG ACCTCCAGTC AAAGTTAAAA ATGGGTCTGG GCATTCTGAT 1260

TTGGCTGAAG GACAACCTGT CCCCATCCCC TAATTGAAAA AGTCGCCCCG AAGCGCTCCT 1320

TCTTCTGAAA GTGAAGATAC TCGCCCATTT ATTCGTTTCA ACATCGAATA TGTTCTCACA 1380

ACCTTTATGG TGCTGCTATG AAGGGCAGTT GTTGCAGAAA TGACACGAAA TTACCTGCTT 1440

TAGCTCGGCG GATTCATGCT TTATTAACAT AAGTGAACGC GAATTAACCG ATGTTAACGT 1500

TTGAAAATGC AAGTTTTTTA GGATCGCCTG CAGAATAAAG CCGCGAATCT TTCGTCGAAA 1560

CAGCCCTTAA CGGAATATGT CGGCAAGGTG GCAAGAATCG TCTGAACGGA GAGCAGAAAC 1620

CTCGAATCCG TTTCATTTAA TAAGGGCAAG TGCGTGCCGG TGCTAAATTG TGGGCCTTTT 1680

TAAGCGCGCC ATATATATAA AGAGAATAAT CCGCAGGAAA TTTTACCAGT TAATGCGTAA 1740

ATCGCTTGAA ATGCCCAGGC GTACCGGTTA TCTCGCCTTT ACCGGAGAGG TGGCCGAGTG 1800

GTCGAAGGCG CTCCCCTGCT AAGGGAGTAG ACCTCAAAAG GGTCTCGTGG GTTCGAATCC 1860

CATCCTCTCC GCCAGTTTTT CCAATATCCC AGCAAATCTT TATGTGTTCG ACGCGCTTGA 1920

TTTCATACGG AATCGGCTTT TACCCCTCGC GCACTGAATC TCTGTTTTTC CAGGCTACGA 1980

ATCCAGAAAA CAAGCAAGCC ATTGATAAGT AATGGCTATT CAAAATTCTG GCGATTCTTG 2040

ACTGGAGGTC AGAAATGAAC ATCAAGAGCC TTCTCCTTGG CTCCGCTGCA GCTCTGGTTG 2100

CAGCTTCCGG CGCTCAGGCT GCCGACGCAA TCGTCGCGCC AGAGCCCGAA GCCGTTGAAT 2160

-continued

```
ATGTCCGCGT TTGCGACGCT TACGGCGCTG GCTACTTCTA CATTCCGGGC ACCGAAACCT   2220
GCCTGCGCGT CCATGGTTAC GTCCGTTACG ACGTAAAGGG CGGCGATGAC GTTTACTCCG   2280
GTACCGACCG CAATGGCTGG GACAAGGGCG CTCGTTTCGC ACTCATGTTC AACACGAATT   2340
CGGAAACCGA ACTCGGCACA CTCGGCACCT ATACTCAGCT GCGCTTCAAC TACACCAGCA   2400
ACAATTCACG TCATGATGGC CAATACGGCG ATTTCAGCGA TGATCGTGAT GTCGCTGATG   2460
GCGGCGTAAG CACCGGCACC GATCTGCAGT TTGCATATAT CACGCTTGGT GGTTTCAAGG   2520
TTGGTATCGA CGAATCCGAA TTCCATACCT TCACCGGTTA CCTCGGTGAT GTCATCAACG   2580
ATGATGTCGT CGCTGCTGGC TCCTACCGCA CCGGCAAGAT CGCCTACACC TTCACCGGCG   2640
GAAACGGCTT CTCGGCTGTG ATCGCTCTCG AACAGGGTGG CGAAGACGTT GACAACGATT   2700
ACACGATCGA CGGTTACATG CCGCACGTTG TTGGCGGCCT GAAATATGCT GGCGGCTGGG   2760
GTTCGATCGC TGGTGTTGTT GCCTATGACT CGGTCATCGA AGAATGGGCT ACAAAGGTTC   2820
GTGGCGACGT CAACATCACC GACCGGTTCT CGGTATGGCT GCAGGGCGCA TATTCGTCCG   2880
CAGCGACGCC GAACCAGAAC TACGGTCAGT GGGGCGGCGA TTGGGCTGTC TGGGGTGGTG   2940
CAAAGTTCAT TGCCCCCGAA AAGGCAACCT TCAATCTGCA GGCTGCGCAT GACGACTGGG   3000
GCAAGACCGC AGTTACCGCC AACGTCGCTT ATCAGCTCGT TCCCGGATTC ACCATTACGC   3060
CGGAAGTTTC CTACACCAAA TTTGGTGGCG AGTGGAAAGA CACCGTTGCT GAAGACAATG   3120
CCTGGGGCGG TATCGTTCGC TTCCAGCGCT CGTTCTAATC AGATCGACGT TAAGCATAGG   3180
GCGCCAACGG TTTCCCGTTG GCGCCGGTTC ATTTGAAACA GCGTTCACGA AAGCGTGAGA   3240
ATCGATTCTT CCGGAATGGG GATTCCAGGC GGATCGACAA TTGAGGGAAT TGCGGGGACG   3300
ACAAAAAGCT GGGGGCAACC GGGGGGTCTT GTAAAGGATT GAGCCAA                  3347
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3208 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Brucella abortus
(B) STRAIN: biovar 1 (S2308)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
CAGGCGATCT TCCGCGACCC CTGTAGAAAG ACTGCGGTCA GCATAAAAAG CAAGCATCTG     60
ATGCTGCACG AGGGCAACAA AAAACCCGGC ATTTCTGCCG GGTTTCTGTA TCCAATCCGT    120
AATGGATTAG AACGAACGCT GGAAGCGAAC GATACCGCCC CAAGCATTGT CTTCAGCAAC    180
GGTGTTCTTC CACTCGCCAC CAAACTTGGT GTAGGAAACT TCCGGCGTAA CGGTGAAGCC    240
AGGAACCAGT TCGTAAGCAA CGTTAGCCGT AACTGCCGTC TTGCCCCAGT CGTCATGCGC    300
AGCCTGCAGG TTGAAGGCAG CCTTCTGCGT AGCCTGATAC TTCAGACCAC CCCAGACAGC    360
CCAATCGCCG CCCCACTGGC CGTAGTTCTG ATCCGGCGTA GCAGCGGACG AATATGCGCC    420
CTGCAACCAA ACCGAGAACT GGTCGGTGAT GTTGACGTCG CCACGAACCT TGGCAGCCCA    480
TTCTTCTATG ACCGAGTCAT AGGCAACAAC ACCAGCGATC GAACCCCAGC CGCCAGCATA    540
CTTCAGGCCG CCAACAACGT CAGGCATGTA GCCGTCGATG TGGTAGTTGG TCGTGCCAGT    600
GTAACCACCG TCGTTGTCGC CACCCTGTTC GAGAGCGATC ACAGCCGAGA AGCCGTTTCC    660
GCCAGTGAAG GTGTACGAGA TCTTGCCGGT GCGGTAGGAG CCAGCCGAGA TCACGTCATC    720
```

-continued

GTTGATGACA TCGCCGAGGT AACCGGTGAA GGTATGGAAT TCCGATTCAT CGATACCAAC	780
GCGCAGACCA CCGAGCTGGA TATACGCGAA CTCCATGACG GTGCCGCTGC TGGTTTCATT	840
ACCATATTTA CCATCTACGC CCGAATTGTT CGCAGCATAG TTGAAGCGCA GTTCGGTGAA	900
GGTCTTGAGG GTGCCGAGTT CGGTTTCCGA ACCGGTGGAA ACGCGGAGTG CGAAACGAGC	960
GCTCTTGTCC CAGCCATTGC GGTCGGTACC GGAGTAAACG TCATCGCCGC CCTTTACGTC	1020
GTAACGGACG TAACCATGGA CGCGCAGGCA GGTTTCGGTG CCCGGAATGT AGAAGTAGCC	1080
AGCGCCGTAA GCGTCGCAAA CGCGGACATA TTCAACGGCT TCGGGCTCTG GCGCGACGAT	1140
TGCGTCGGCA GCCTGAGCGC CGGAAGCTGC AACCAGAGCT GCAGCGGAGC CAAGGAGAAG	1200
GCTCTTGATG TTCATTTCTG ACCTCCAGTC AAAGTTAAAA ATGGGTCTGG GCATTCTGAT	1260
TTGGCTGAAG GACAACCTGT CCCCATCCCC TAATTGAAAA AGTCGCCCCG AAGCGCTCCT	1320
TCTTCTGAAA GTGAAGATAC TCGCCCATTT ATTCGTTTCA ACATCGAATA TGTTCTCACA	1380
ACCTTTATGG TGCTGCTATG AAGGGCAGTT GTTGCAGAAA TGACACGAAA TTACCTGCTT	1440
TAGCTCGGCG GATTCATGCT TTATTAACAT AAGTGAACGC GAATTAACCG ATGTTAACGT	1500
TTGAAAATGC AAGTTTTTTA GGATCGCCTG CAGAATAAAG CCGCGAATCT TTCGTCGAAA	1560
CAGCCCTTAA CGGAATATGT CGGCAAGGTG GCAAGAATCG TCTGAACGGA GAGCAGAAAC	1620
CTCGAATCCG TTTCATTTAA TAAGGGCAAG TGCGTGCCGG TGCTAAATTG TGGGCCTTTT	1680
TAAGCGCGCC ATATATATAA AGAGAATAAT CCGCAGGAAA TTTTACCAGT TAATGCGTAA	1740
ATCGCTTGAA ATGCCCAGGC GTACCGGTTA TCTCGCCTTT ACCGGAGAGG TGGCCGAGTG	1800
GTCGAAGGCG CTCCCCTGCT AAGGGAGTAG ACCTCAAAAG GGTCTCGTGG GTTCGAATCC	1860
CATCCTCTCC GCCAGTTTTT CCAATATCCC AGCAAATCTT TATGTGTTCG ACGCGCTTGA	1920
TTTCATACGG AATCGGCTTT TACCCCTCGC GCACTGAATC TCTGTTTTTC CAGGCTACGA	1980
ATCCAGAAAA CAAGCAAGCC ATTGATAAGT AATGGCTATT CAAAATTCTG GCGATTCTTG	2040
ACTGGAGGTC AGAAATGAAC ATCAAGAGCC TTCTCCTTGG CTCCGCTGCA GCTCTGGTTG	2100
CAGCTTCCGG CGCTCAGGCT GCCGACGCAA TCGTCGCGCC AGAGCCCGAA GCCGTTGAAT	2160
ATGTCCGCGT TTGCGACGCT TACGGCGCTG GCTACTTCTA CATTCCGGGC ACCGAAACCT	2220
GCCTGCGCGT CCATGGTTAC GTCCGTTACG ACGTAAAGGG CGGCGATGAC GTTTACTCCG	2280
GTACCGACCG CAATGGCTGG GACAAGGGCG CTCGTTTCGC ACTCATGTTC AACACGAATT	2340
CGGAAACCGA ACTCGGCACA CTCGGCACCT ATACTCAGCT GCGCTTCAAC TACACCAGCA	2400
ACAATTCACG TCATGATGGC CAATACGGCG ATTTCAGCGA TGATCGTGAT GTCGCTGATG	2460
GCGGCGTAAG CACCGGCAAG ATCGCCTACA CCTTCACCGG CGGAAACGGC TTCTCGGCTG	2520
TGATCGCTCT CGAACAGGGT GGCGAAGACG TTGACAACGA TTACACGATC GACGGTTACA	2580
TGCCGCACGT TGTTGGCGGC CTGAAATATG CTGGCGGCTG GGGTTCGATC GCTGGTGTTG	2640
TTGCCTATGA CTCGGTCATC GAAGAATGGG CTACAAAGGT TCGTGGCGAC GTCAACATCA	2700
CCGACCGGTT CTCGGTATGG CTGCAGGGCG CATATTCGTC CGCAGCGACG CCGAACCAGA	2760
ACTACGGTCA GTGGGGCGGC GATTGGGCTG TCTGGGGTGG TGCAAAGTTC ATTGCCCCCG	2820
AAAAGGCAAC CTTCAATCTG CAGGCTGCGC ATGACGACTG GGGCAAGACC GCAGTTACCG	2880
CCAACGTCGC TTATCAGCTC GTTCCCGGAT TCACCATTAC GCCGGAAGTT TCCTACACCA	2940
AATTTGGTGG CGAGTGGAAA GACACCGTTG CTGAAGACAA TGCCTGGGGC GGTATCGTTC	3000
GCTTCCAGCG CTCGTTCTAA TCAGATCGAC GTTAAGCATA GGGCGCCAAC GGTTTCCCGT	3060
TGGCGCCGGT TCATTTGAAA CAGCGTTCAC GAAAGCGTGA GAATCGATTC TTCCGGAATG	3120
GGGATTCCAG GCGGATCGAC AATTGAGGGA ATTGCGGGGA CGACAAAAAG CTGGGGGCAA	3180

-continued

CCGGGGGGTC TTGTAAAGGA TTGAGCCA 3208

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3346 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Brucella canis

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CAGGCGATCT TCCGCGACCC CTGTAGAAAG ACTGCGGTCA GCATAAAAAG CAAGCATCTG 60
ATGCTGCACG AGGGCAACAA AAAACCCGGT ATTTCTGCCG GGTTTCTGTA TCCAATCCGT 120
AATGGATTAG AACGAACGCT GGAAGCGAAC GATACCGCCC CAAGCATTGT CTTCAGCAAC 180
GGTGTTCTTC CACTCGCCAC CAAACTTGGT GTAGGAAACT TCCGGCGTAA CGGTGAAGCC 240
AGGAACCAGT TCGTAAGCAA CGTTAGCCGT AACTGCCGTC TTGCCCCAGT CGTCATGCGC 300
AGCCTGCAGG TTGAAGGCAG CCTTCTGCGT AGCCTGATAC TTCAGACCAC CCCAGACAGC 360
CCAATCGCCG CCCCACTGGC CGTAGTTCTG ATCCGGCGTA GCAGCGGACG AATATGCGCC 420
CTGCAACCAA ACCGAGAACT GGTCGGTGAT GTTGACGTCG CCACGAACCT TGGCAGCCCA 480
TTCTTCGATG ACCGAGTCAT AGGCAACAAC ACCAGCGATC GAACCCCAGC CGCCAGCATA 540
CTTCAGGCCG CCAACAACGT CAGGCATGTA GCCGTCGATG TGGTAGTTGG TCGTGCCAGT 600
GTAACCACCG TCGTTGTCGC CACCCTGTTC GAGAGCGATC ACAGCCGAGA AGCCGTTTCC 660
GCCAGTGAAG GTGTACGAGA TCTTGCCGGT GCGGTAGGAG CCAGCCGAGA TCACGTCATC 720
GTTGATGACA TCGCCGAGGT AACCGGTGAA GGTATGGAAT TCCGATTCAT CGATACCAAC 780
GCGCAGACCA CCGAGCTGGA TATACGCGAA CTCCATGACG GTGCCGCTGC TGGTTTCATT 840
ACCATATTTA CCATCTACGC CCGAATTGTT CGCAGCATAG TTGAAGCGCA GTTCGGTGAA 900
GGTCTTGAGG GTGCCGAGTT CGGTTTCCGA ACCGGTGGAA ACGCGGAGTG CGAAACGAGC 960
GCTCTTGTCC CAGCCTTTAC GATCCGAGCC GGTATAAACG TCGTCGCCGC CCTTTACGTC 1020
GTAACGGACG TAACCATGGA CGCGCAGGCA GGTTTCGGTG CCCGGAATGT AGAAGTAGCC 1080
AGCGCCGTAA GCGTCGCAAA CGCGGACATA TTCAACGGCT TCGGGCTCTG GCGCGACGAT 1140
TGCGTCGGCA GCCTGAGCGC CGGAAGCTGC AACCAGAGCT GCAGCGGAGC CAAGGAGAAG 1200
GCTCTTGATG TTCATTTCTG ACCTCCAGTC AAAGTTAAAA ATGGGTCTGG GCATTCTGAT 1260
TTGGCTGAAG GACAACCTGT CCCCATCCCC TAATTGAAAA AGTCGCCCCG AAGCGCTCCT 1320
TCTTCTGAAA GTGAAGATAC TCGCCCATTT ATTCGTTTCA ACATCGAATA TGTTCTCACA 1380
ACCTTTACGG TGCTGCTATG AAGGGCAGTT GTTGCTGAAA TGACACAAAA TTACCTGCTT 1440
TAGCTCGGCG GATTCATGCT TTATTAACAT AAGTAAACGC GAATTAACCG ATGTTAACGT 1500
TTGAAAATGC AAGTTTTTTA GGATCGCCTA CCGAATAAAG CCGCGAATCT TTCGTCGAAA 1560
CAGCCCTTAA CGGAATATGT CGGCAAGGTG GCAAGAATCG TCTGAACGGA GAGCAGAAAC 1620
CTCGAATCCG TTTCATTTAA TAAGGGCAAG TGCGTGCCGG TGCTAAATTG TGGGCCTTTT 1680
TAAGCGCGCT ATATATATAA AGAGAATAAT CCGCAGGAAA TTTTACCAGT TAATGCGTAA 1740
ATCGCTTGAA ATGCCCAGGC GTACCGGTTA TCTCGCCTTT ACCGGAGAGG TGGCCGAGTG 1800
GTCGAAGGCG CTCCCCTGCT AAGGGAGTAG ACCTCAAAAG GGTCTCGTGG GTTCGAATCC 1860
CATCCTCTCC GCCAGTTTTT CCAATATCCC AGCAAATCTT TATGTGTTCG ACGCGCTTGA 1920

-continued

```
TTTCATACGG AATCGGCTTT TACCCCTCGC GCACTGAATC TCTGTTTTTC CAGGCTACGA   1980
ATCCAGAAAA CAAGCAAGCC ATTGATAAGT AATGGCTATT CAAAATTCTG GCGATTCTTG   2040
ACTGGAGGTC AGAAATGAAC ATCAAGAGCC TTCTCCTTGG CTCCGCTGCA GCTCTGGTTG   2100
CAGCTTCCGG CGCTCAGGCT GCCGACGCAA TCGTCGCGCC AGAGCCCGAA GCCGTTGAAT   2160
ATGTCCGCGT TTGCGACGCT TACGGCGCTG GCTACTTCTA CATTCCGGGC ACCGAAACCT   2220
GCCTGCGCGT CCATGGTTAC GTCCGTTACG ACGTAAAGGG CGGCGACGAC GTTTATACCG   2280
GCTCGGATCG TAAAGGCTGG GACAAGGGCG CTCGTTTCGC ACTCATGTTC AACACGAATT   2340
CGGAAACCGA ACTCGGCACA CTCGGCACCT ATACTCAGCT GCGCTTCAAC TACACCAGCA   2400
ACAATTCACG TCATGATGGC CAATACGGCG ATTTCAGCGA TGATCGTGAT GTCGCTGATG   2460
GCGGCGTAAG CACCGGCACC GATCTGCAGT TTGCATATAT CACGCTTGGT GGTTTCAAGG   2520
TTGGTATCGA CGAATCCGAA TTCCATACCT TCACCGGTTA CCTCGGTGAT GTCATCAACG   2580
ATGATGTCGT CGCTGCTGGC TCCTACCGCA CCGGCAAGAT CGCCTACACC TTCACCGGCG   2640
GAAACGGCTT CTCGGCTGTG ATCGCTCTCG AACAGGGTGG CGAAGACGTT GACAACGATT   2700
ACACGATCGA CGGTTACATG CCGCACGTTG TTGGCGGCCT GAAATATGCT GGCGGCTGGG   2760
GTTCGATCGC TGGTGCTGTT GCCTATGACC CGGTCATCGA AGAATGGGCT ACAAAGGTTC   2820
GTGGCGACGT CAACATCACC GACCGGTTCT CGGTATGGCT GCAGGGCGCA TATTCGTCCG   2880
CAGCGACGCC GAACCAGAAC TACGGTCAGT GGGGCGGCGA TTGGGCTGTC TGGGGTGGTG   2940
CAAAGTTCAT TGCCACCGAA AAGGCAACCT TCAATCTGCA GGCTGCGCAT GACGACTGGG   3000
GCAAGACCGC AGTTACCGCC AACGTCGCTT ATCAGCTCGT TCCCGGATTC ACCATTACGC   3060
CGGAAGTTTC CTACACCAAA TTTGGTGGCG AGTGGAAAGA CACCGTTGCT GAAGACAATG   3120
CCTGGGGCGG TATCGTTCGC TTCCAGCGCT CGTTCTAATC AGATCGACGT TAAGCATAGG   3180
GCGCCAACGG TTTCCCGTTG GCGCCGGTTC ATTTGAAACA GCGTTCACGA AAGCGTGAGA   3240
ATCGATTCTT CCGGAATGGG GATTCCAGGC GGATCGACAA TTGAGGGAAT TGCGGGGACG   3300
ACAAAAAGCT GGGGGCAACC GGGGGGTCTT GTAAAGGATT GAGCCA                   3346
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3346 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Brucella neotomae

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CAGGCGATCT TCCGCGACCC CTGTAGAAAG ACTGCGGTCA GCATAAAAAG CAAGCATCTG     60
ATGCTGCACG AGGGCAACAA AAAACCCGGC ATTTCTGCCG GGTTTCTGTA TCCAATCCGT    120
AATGGATTAG AACGAACGCT GGAAGCGAAC GATACCGCCC CAAGCATTGT CTTCAGCAAC    180
GGTGTTCTTC CACTCGCCAC CAAACTTGGT GTAGGAAACT TCCGGAGCAA CGGTGAAGCC    240
AGGAACCAGT TCGTAAGCAA CGTTAGCCGT AACTGCCGTC TTGCCCCAGT CGTCATGCGC    300
AGCCTGCAGA TTGAAGGTTG CCTTTTCGGT GGCAATGAAC TTTGCACCAC CCCAGACAGC    360
CCAATCGCCG CCCCACTGAC CGTAGTTCTG GTTCGGCGTC GCTGCGGACG AATATGCGCC    420
CTGCAACCAA ACCGAGAACT GGTCGGTGAT GTTGACGTCG CCACGAACCT TGGCAGCCCA    480
TTCTTCGATG ACCGAGTCAT AGGCAACAAC ACCAGCGATC GAACCCCAGC CGCCAGCATA    540
```

-continued

CTTCAGGCCG CCAACAACGT CAGGCATGTA GCCGTCGATG TGGTAGTTGG TCGTGCCAGT 600

GTAACCACCG TCGTTGTCGC CACCCTGTTC GAGAGCGATC ACAGCCGAGA AGCCGTTTCC 660

GCCAGTGAAG GTGTACGAGA TCTTGCCGGT GCGGTAGGAG CCAGCCGAGA TCACGTCATC 720

GTTGATGACA TCGCCGAGGT AACCGGTGAA GGTATGGAAT TCCGATTCAT CGATACCAAC 780

GCGCAGACCA CCGAGCTGGA TATACGCGAA CTCCATGACG GTGCCGCTGC TGGTTTCATT 840

ACCATATTTA CCATCTACGC CCGAATTGTT CGCAGCATAG TTGAAGCGCA GTTCGGTGAA 900

GGTCTTGAGG GTGCCGAGTT CGGTTTCCGA ACCGGTGGAA ACGCGGAGTG CGAAACGAGC 960

GCTCTTGTCC CAGCCATTGC GGTCGGTACC GGAGTAAACG TCATCGCCGC CCTTTACGTC 1020

GTAACGGACG TAACCATGGA CGCGCAGGCA GGTTTCGGTG CCCGGAATGT AGAAGTAGCC 1080

AGCGCCGTAA GCGTCGCAAA CGCGGACATA TTCAACGGCT TCGGGCTCTG GCGCGACGAT 1140

TGCGTCGGCA GCCTGAGCGC CGGAAGCTGC AACCAGAGCT GCAGCGGAGC CAAGGAGAAG 1200

GCTCTTGATG TTCATTTCTG ACCTCCAGTC AAAGTTAAAA ATGGGTCTAG GCATTCTGAT 1260

TTGGCTGAAG GACAACCTGT CCCCATCCCC TAATTGAAAA AGTCGCCCCG AAGCGCTCCT 1320

TCTTCTGAAA GTGAAGATAC TCGCCCATTT ATTCGTTTCA ACATCGAATA TGTTCTCACA 1380

ACCTTTACGG TGCTGCTATG AAGGGCAGTT GTTGCAGAAA TGACACGAAA TTACCTGCTT 1440

TAGCTCGGCG GATTCATGCT TTATTAACAT AAGTGAACGC GAATTAACCG ATGTTAACGT 1500

TTGAAAATGC AAGTTTTTTA GGATCGCCTG CCGAATAAAG CCGCAAATCT TTCGTCGAAA 1560

CAGCCCTTAA CGGAATATGT CGGCAAGGTG GCAAGAATCG TCTGAACGGA GAGCAGAAAC 1620

CTCGAATCCG TTTCATTTAA TAAGGGCAAG TGCGTGCCGG TGCTAAATTG TGGGCCTTTT 1680

TAAGCGCGCC ATATATATAA AGAGAATAAT CCGCAGGAAA TTTTACCAGT TAATGCGTAA 1740

ATCGCTTGAA ATGCCCAGGC GTACCGGTTA TCTCGCCTTT ACCGGAGAGG TGGCCGAGTG 1800

GTCGAAGGCG CTCCCCTGCT AAGGGAGTAG ACCTCAAAAG GGTCTCGTGG GTTCGAATCC 1860

CATCCTCTCC GCCAGTTTTT CCAATATCCC AGCAAATCTT TATGTGTTCG ACGCGCTTGA 1920

TTTCATACGG AATCGGCTTT TACCCCTCGC GCACTGAATC TCTGTTTTTC CAGGCTACGA 1980

ATCCAGAAAA CAAGCAAGCC ATTGATAAGT AATGGCTATT CAAAATTCTG GCGATTCTTG 2040

ACTGGAGGTC AGAAATGAAC ATCAAGAGCC TTCTCCTTGG CTCCGCTGCA GCTCTGGTTG 2100

CAGCTTCCGG CGCTCAGGCT GCCGACGCAA TCGTCGCGCC GGAGCCCGAA GCCGTTGAAT 2160

ATGTCCGCGT TTGCGACGCT TACGGCGCTG GCTACTTCTA CATTCCGGGC ACCGAAACCT 2220

GCCTGCGCAT CAGCGGCTAC GTCCGTTACG ACGTAAAGGG CGGCGACGAC GTTTATACCG 2280

GCTCGGATCG TAAAGGCTGG GACAAGGGCG CTCGTTTCGC ACTCATGTTC AACACGAATT 2340

CGGAAACCGA ACTCGGCACA CTCGGCACCT ATACTCAGCT GCGCTTCAAC TACACCAGCA 2400

ACAATTCACG TCATGATGGC CAATACGGCG ATTTCAGCGA TGATCGTGAT GTCGCTGATG 2460

GCGGCGTAAG CACCGGCACC GATCTGCAGT TTGCATATAT CACGCTTGGT GGTTTCAAGG 2520

TTGGTATCGA CGAATCCGAA TTCCATACCT TCACCGGTTA CCTCGGTGAT GTCATCAACG 2580

ATGATGTCGT CGCTGATGGC TCCTACCGCA CCGGCAAGAT CGCCTACACC TTCACCGGCG 2640

GAAACGGCTT CCCGGCTGTG ATCGCTCTCG AACAGGGTGG CGAAGACGTT GACAACGATT 2700

ACACGATCGA CGGTTACATG CCGCACGTTG TTGGCGGCCT GAAATATGCT GGCGGCTGGG 2760

GTTCGATCGC TGGTGTTGTT GCCTATGACT CGGTCATCGA AGAATGGGCT ACAAAGGTTC 2820

GTGGCGACGT CAACATCACC GACCGGTTCT CGGTATGGCT GCAGGGCGCA TATTCGTCCG 2880

CAGCGACGCC GAACCAGAAC TACGGTCAGT GGGGCGGCGA TTGGGCTGTC TGGGGTGGTG 2940

CAAAGTTCAT TGCCACCGAA AAGGCAACCT TCAATCTGCA GGCTGCGCAT GACGACTGGG 3000

-continued

```
GCAAGACGGC AGTTACGGCT AACGTTGCTT ACGAACTGGT TCCTGGCTTC ACCGTTACGC   3060
CGGAAGTTTC CTACACCAAA TTTGGTGGCG AGTGGAAAGA CACCGTTGCT GAAGACAATG   3120
CCTGGGGCGG TATCGTTCGC TTCCAGCGCT CGTTCTAATC AGATCGACGT TAAGCATAGG   3180
GCGCCAACGG TTTCCCGTTG GCGCCGGTTC ATTTGAAACA GCGTTCACGA AAGCGTGAGA   3240
ATCGATTCTT CCGGAATGGG GATTCCAGGC GGATCGACAA TTGAGGGAAT TGCGGGGACG   3300
ACAAAAAGCT GGGGGCAACC GGGGGGTCTT GTAAAGGATT GAGCCA                   3346
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3361 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Brucella ovis

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
CAGGCGATCT TCCGCGACCC CTGTAGAAAG ACTGCGGTCA GCATAAAAAG CAAGCATCTG     60
ATGCTGCACG AGGGCAACAA AAAACCCGGC ATTTCTGCCG GGTTTCTGTA TCCAATCCGT    120
AATGGATTAG AACGAACGCT GGAAGCGAAC GATACCGCCC CAAGCATTGT CTTCAGCAAC    180
GGTGTTCTTC CACTCGCCAC CAAACTTGGT GTAGGAAACT TCCGGCGTAA CGGTGAAGCC    240
AGGAACCAGT TCGTAAGCAA CGTTAGCCGT AACTGCCGTC TTGCCCCAGT CGTCATGCGC    300
AGCCTGCAGG TTGAAGGCAG CCTTCTGCGT AGCCTGATAC TTCAGACCAC CCCAGACAGC    360
CCAATCGCCG CCCCACTGAC CGTAGTTCTG GTTCGGCGTC GCTGCGGACG AATATGCGCC    420
CTGCAGCCAT ACCGAGAACC GGTCGGTGAT GTTGACGTCG CCACGAACCT TTGTAGCCCA    480
TTCTTCGATG ACCGAGTCAT AGGCAACAAC ACCAGCGATC GAACCCCAGC CGCCAGCATA    540
TTTCAGGCCG CCAACAACGT GCGGCATGTA ACCGTCGATC GTGTAATCGT TGTCAACGTC    600
TTCGCCACCC TGTTCGAGAG CGATCACAGC CGAGAAGCCG TTTCCGCCGG TGAAGGTGTA    660
GGCGATCTTG CCGGTGCGGT AGGAGCCAGC CGAGATCACG TCATCGTTGA TGATATCACC    720
GAGGTAACCG GTGAAGGTAT GGAATTCGGA TTCGTCGATA CCAACCTTGA AACCACCAAG    780
CGTGATATAT GCAAACTGCA GATCGGTGCC GGTGCTTACG CTGCCATCAG CGACATCACG    840
ATCATCGCTG AAATCGCCGT ATTGGCCATC ATGACGTGAA TTGTTGCTGG TGTAGTTGAA    900
GCGCAGCGTA GTATAGGTGC CGAGTGTGCC GAGTTCGGTT TCCGAATTCG TGTTGAACAT    960
GGAGTGCAAA ACGAGCACCT TGTCCCAGCC TTTACGATCC GAGCCGGTAT AAACGTCGTC   1020
GCCGCCCTTT ACGTCGTAAC GGACGTAGCC GCTGATGCGC AGGCAGGTTT CGGTGCCCGG   1080
AATGTAGAAG TAGCCAGCGC CATAAGCGTC GCAAACGCGG ACATATTCAA CGGCTTCGGG   1140
CTCTGGCGCG ACGATTGCGT CGGCAGCTTG AGCGCCGGAA GCTGCAACCA GAGCTGCAGC   1200
GGAGCCAAGG AGAAGGCTCT TGATGTTCAT TTCTGACCTC CAGTCAAAGT TAAAAATGGG   1260
TCTGGGCATT CTGATTTGGC TGAAGGACAA CCTGTCCCCA TCCCCTAATT GAAAAAGTCG   1320
CCCCGAAGCG CTCCTTCTTC TGAAAGTGAA GATACTCGCC CATTTATTCG TTTCAACATC   1380
GAATATGTTC TCACAACCTT TACGGTGCTG CTATGAAGGG CAGTTATTGC AGAAATGACA   1440
CGAAATTACC TGCTTTAGCT CGGCGGATTC ATGCTTTATT AACATAAGTG AACGCGAATT   1500
AACCGATGTT AACGTTTGAA AATGCAAGTT TTTTAGGATC GCCTGCCGAA TAAAGCCGCG   1560
AATCTTTCGT CGAAACAGCC CTTAACGGAA TATGTCGGCA AGGTGGCAAG AATCGTCTGA   1620
```

-continued

ACGGAGAGCA GAAACCTCGA ATCCGTTTCA TTTAATAAGG GCAAGTGCGT GCCGGTGCTA 1680

AATTGTGGGC CTTTTTAAGC GCGCCATATA TATAAAGAGA ATAATCCGCA GGAAATTTTA 1740

CCAGTTAATG CGTAAATCGC TTGAAATGCC CAGGCGTACC GGTTATCTCG CCTTTACCGG 1800

AGAGGTGGCC GAGTGGTCGA AGGCGCTCCC CTGCTAAGGG AGTAGACCTC AAAAGGGTCT 1860

CGTGGGTTCG AATCCCATCC TCTCCGCCAG TTTTTCCAAT ATCCCAGCAA ATCTTTATGT 1920

GTTCGACGCG CTTGATTTCA TACGGAATCG GCTTTTACCC CTCGCGCACT GAATCTCTGT 1980

TTTTCCAGGC TACGAATCCA GAAAACAAGC AAGCCATTGA TAAGTAATGG CTATTCAAAA 2040

TTCTGGCAAT TCTTGACTGG AGGTCAGAAA TGAACATCAA GAGCCTTCTC CTTGGCTCCG 2100

CTGCAGCTCT GGTTGCAGCT TCCGGCGCTC AAGCTGCCGA CGCAATCGTC GCGCCAGAGC 2160

CCGAAGCCGT TGAATATGTC CGCGTTTGCG ACGCTTATGG CGCTGGCTAC TTCTACATTC 2220

CGGGCACCGA AACCTGCCTG CGCATCAGCG GCTACGTCCG TTACGACGTA AAGGGCGGCG 2280

ACGACGTTTA TACCGGCTCG GATCGTAAAG GCTGGGACAA GGGTGCTCGT TTTGCACTCA 2340

TGTTCAACAC GAATTCGGAA ACCGAACTCG GCACACTCGG CACCTATACT CAGCTGCGCT 2400

TCAACTACAC CAGCAACAAT TCACGTCATG ATGGCCAATA CGGCGATTTC AGCGATGATC 2460

GTGATGTCGC TGATGGCAGC GTAAGCACCG GCACCGATCT GCAGTTTGCA TATATCACGC 2520

TTGGTGGTTT CAAGGTTGGT ATCGACGAAT CCGAATTCCA TACCTTCACC GGTTACCTCG 2580

GTGATATCAT CAACGATGAC GTGATCTCGG CTGGCTCCTA CCGCACCGGC AAGATCGCCT 2640

ACACCTTCAC CGGCGGAAAC GGCTTCTCGG CTGTGATCGC TCTCGAACAG GGTGGCGAAG 2700

ACGTTGACAA CGATTACACG ATCGACGGTT ACATGCCGCA CGTTGTTGGC GGCCTGAAAT 2760

ATGCTGGCGG CTGGGGTTCG ATCGCTGGTG TTGTTGCCTA TGACTCGGTC ATCGAAGAAT 2820

GGGCTACAAA GGTTCGTGGC GACGTCAACA TCACCGACCG GTTCTCGGTA TGGCTGCAGG 2880

GCGCATATTC GTCCGCAGCG ACGCCGAACC AGAACTACGG TCAGTGGGGC GGCGATTGGG 2940

CTGTCTGGGG TGGTGCAAAG TTCATTGCCA CCGAAAAGGC AACCTTCAAT CTGCAGGCTG 3000

CGCATGACGA CTGGGGCAAG ACCGCAGTTA CCGCCAACGT CGCTTATCAG CTCGTTCCCG 3060

GATTCACCAT TACGCCGGAA GTTTCCTACA CCAAATTTGG TGGCGAGTAG AAAGACACCG 3120

TTGCTGAAGA CAATGCCTGG GGCGGTATCG TTCGTTTCCA GCGCTCGTTC TAATCAGATC 3180

GACGTTAAGC ATAGGGCGCC AACGGTTTCC CGTTGGCGCC GGTTCATTTG AAACAGCGTT 3240

CACGAAAGCG TGAGAATCGA TTCTTCCGGA ATGGGGATTC CAGGCGGATC GACAATTGAG 3300

GGAATTGCGG GGACGACAAA AAGCTGGGGG CAACCGGGGG GTCTTGTAAA GGATTGAGCC 3360

A 3361

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3345 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Brucella melitensis
(B) STRAIN: biovar 1

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AGGCGATCTT CCGCGACCCC TGTAGAAAGA CTGCGGTCAG CATAAAAAGC AAGCATCTGA 60

TGCTGCACGA GGGCAACAAA AAACCCGGCA TTTCTGCCGG GTTTCTGTAT CCAATCCGTA 120

ATGGATTAGA ACGAACGCTG GAAGCGAACG ATACCGCCCC AAGCATTGTC TTCAGCAACA 180

-continued

```
GTGTTCTTCC ACTCGCCACC AAACTTGGTG TAGGAAACTT CCGGCGTAAC GGTGAAGCCA   240
GGAACCAGTT CGTAAGCAAC GTTAGCCGTA ACTGCCGTCT TGCCCCAGTC GTCATGCGCA   300
GCCTGCAGGT TGAAGGCAGC CTTCTGCGTA GCCTGATACT TCAGACCACC CCAGACAGCC   360
CAATCGCCGC CCCACTGGCC GTAGTTCTGA TCCGGCGTAG CAGCGGACGA ATATGCGCCC   420
TGCAACCAAA CCGAGAACTG GTCGGTGATG TTGACGTCGC CACGAACCTT GGCAGCCCAT   480
TCTTCGATGA CCGAGTCATA GGCAACAACA CCAGCGATCG AACCCCAGCC GCCAGCATAC   540
TTCAGGCCGC CAACAACGTC AGGCATGTAG CCGTCGATGT GGTAGTTGGT CGTGCCAGTG   600
TAACCACCGT CGTTGTCGCC ACCCTGTTCG AGAGCGATCA CAGCCGAGAA GCCGTTTCCG   660
CCAGTGAAGG TGTACGAGAT CTTGCCGGTG CGGTAGGAGC CAGCCGAGAT CACGTCATCG   720
TTGATGACAT CGCCGAGGTA ACCGGTGAAG GTATGGAATT CCGATTCATC GATACCAACG   780
CGCAGACCAC CGAGCTGGAT ATACGCGAAC TCCATGACGG TGCCGCTGCT GGTTTCATTA   840
CCATATTTAC CATCTACGCC CGAATTGTTC GCAGCATAGT TGAAGCGCAG TTCGGTGAAG   900
GTCTTGAGGG TGCCGAGTTC GGTTTCCGAA CCGGTGGAAA CGCGGAGTGC GAAACGAGCG   960
CCCTTGTCCC AGCCATTGCG GTCGGTACCG GAGTAAACGT CATCGCCGCC CTTTACGTCG  1020
TAACGGACGT AACCATGGAC GCGCAGGCAG GTTTCGGTGC CCGGAATGTA GAAGTAGCCA  1080
GCGCCGTAAG CGTCGCAAAC GCGGACATAT TCAACGGCTT CGGGCTCTGG CGCGACGATT  1140
GCGTCGGCAG CCTGAGCGCC GGAAGCTGCA ACCAGAGCTG CAGCGGAGCC AAGGAGAAGG  1200
CTCTTGATGT TCATTTCTGA CCTCCAGTCA AAGTTAAAAA TGGGTCTGGG CATTCTGATT  1260
TGGCTGAAGG ACAACCTGTC CCCATCCCCT AATTGAAAAA GTCGCCCCGA AGCGCTCCTT  1320
CTTCTGAAAG TGAAGATACT CGCCCATTTA TTCGTTTCAA CATCGAATAT GTTCTCACAA  1380
CCTTTACGGT GCTGCTATGA AGGGCAGTTA TTGCAGAAAT GACACGAAAT TACCTGCTTT  1440
AGCTCGGCGG ATTCATGCTT TATTAACATA AGTGAACGCG AATTAACCGA TGTTAACGTT  1500
TGAAAATGCA AGTTTTTTAG GATCGCCTGC CGAATAAAGC CGCGGATCTT TCGTCGAAAC  1560
AGCCCTTAAC GGAATATGTC GGCAAGGTGG CAAGAATCGT CTGAACGGAG AGCAGAAACC  1620
TCGAATCCGT TTCATTTAAT AAGGGCAAGT GCGTGCCGGT GCTAAATTGT GGGCCTTTTT  1680
AAGCGCGCCA TATATATAAA GAGAATAATC CGCAGGAAAT TTTACCAGTT AATGCGTAAA  1740
TCGCTTGAAA TGCCCAGGCG TACCGGTTAT CTCGCCTTTA CCGGAGAGGT GGCCGAGTGG  1800
TCGAAGGCGC TCCCCTGCTA AGGGAGTAGA CCTCAAAAGG GTCTCGTGGG TTCGAATCCC  1860
ATCCTCTCCG CCAGTTTTTC CAATATCCCA GCAAATCTTT ATGTGTTCGA CGCGCTTGAT  1920
TTCATACGGA ATCGGCTTTT ACCCCTCGCG CACTGAATCT CTGTTTTTCC AGGCTACGAA  1980
TCCAGAAAAC AAGCAAGCCA TTGATAAGTA ATGGCTATTC AAAATTCTGG CGATTCTTGA  2040
CTGGAGGTCA GAAATGAACA TCAAGAGCCT TCTCCTTGGC TCCGCCGCAG CTCTGGTTGC  2100
AGCTTCCGGC GCTCAGGCTG CCGACGCAAT CGTCGCGCCA GAGCCCGAAG CCGTTGAATA  2160
TGTCCGCGTT TGCGACGCTT ACGGCGCTGG CTACTTCTAC ATTCCGGGCA CCGAAACCTG  2220
CCTGCGCGTC CATGGTTACG TCCGTTACGA CGTAAAGGGC GGCGATGACG TTTACTCCGG  2280
TACCGACCGC AATGGCTGGG ACAAGGGCGC TCGTTTCGCA CTCATGTTCA ACACGAATTC  2340
GGAAACCGAA CTCGGCACAC TCGGCACCTA TACTCAGCTG CGCTTCAACT ACACCAGCAA  2400
CAATTCACGT CATGATGGCC AATACGGCGA TTTCAGCGAT GATCGTGATG TCGCTGATGG  2460
CGGCGTAAGC ACCGGCACCG ATCTGCAGTT TGCATATATC ACGCTTGGTG GTTTCAAGGT  2520
TGGTATCGAC GAATCCGAAT TCCATACCTT CACCGGTTAC CTCGGTGATG TCATCAACGA  2580
TGATGTCGTC GCTGCTGGCT CCTACCGCAC CGGCAAGATC GCCTACACCT TCACCGGCGG  2640
```

-continued

AAACGGCTTC TCGGCTGTGA TCGCTCTCGA ACAGGGTGGC GAAGACGTTG ACAACGATTA 2700

CACGATCGAC GGTTACATGC CGCACGTTGT TGGCGGCCTG AAATATGCTG GCGGCTGGGG 2760

TTCGATCGCT GGTGTTGTTG CCTATGACTC GGTCATCGAA GAATGGGCTA CAAAGGTTCG 2820

TGGCGACGTC AACATCACCG ACCGGTTCTC GGTATGGCTG CAGGGCGCAT ATTCGTCCGC 2880

AGCGACGCCG AACCAGAACT ACGGTCAGTG GGGCGGCGAT TGGGCTGTCT GGGGTGGTGC 2940

AAAGTTCATT GCCACCGAAA AGGCAACCTT CAATCTGCAG GCTGCGCATG ACGACTGGGG 3000

CAAGACCGCA GTTACCGCCA ACGTCGCTTA TCAGCTCGTT CCCGGATTCA CCATTACGCC 3060

GGAAGTTTCC TACACCAAAT TTGGTGGCGA GTGGAAAGAC ACCGTTGCTG AAGACAATGC 3120

CTGGGGCGGT ATCGTTCCTT TCCAGCGCTC GTTCTAATCA GATCGACGTT AAGCATAGGG 3180

CGCCAACGGT TTCCCGTTGG CGCCGGTTCA TTTGAAACAG CGTTCACGAA AGCGTGAGAA 3240

TCGATTCTTC CGGAATGGGG ATTCCAGGCG GATCGACAAT TGAGGGAATT GCGGGGACGA 3300

CAAAAAGCTG GGGGCAACCG GGGGGTCTTG TAAAGGATTG AGCCA 3345

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3347 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Brucells suis
(B) STRAIN: biovar 1

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CAGGCGATCT TCCGCGACCC CTGTAGAAAG ACTGCGGTCA GCATAAAAAG CAAGCATCTG 60

ATGCTGCACG AGGGCAACAA AAAACCCGGT ATTTCTGCCG GGTTTCTGTA TCCAATCCGT 120

AATGGATTAG AACGAACGCT GGAAGCGAAC GATACCGCCC CAAGCATTGT CTTCAGCAAC 180

GGTGTTCTTC CACTCGCCAC CAAACTTGGT GTAGGAAACT TCCGGCGTAA CGGTGAAGCC 240

AGGAACCAGT TCGTAAGCAA CGTTAGCCGT AACTGCCGTC TTGCCCCAGT CGTCATGCGC 300

AGCCTGCAGG TTGAAGGCAG CCTTCTGCGT AGCCTGATAC TTCAGACCAC CCCAGACAGC 360

CCAATCGCCG CCCCACTGGC CGTAGTTCTG ATCCGGCGTA GCAGCGGACG AATATGCGCC 420

CTGCAACCAA ACCGAGAACT GGTCGGTGAT GTTGACGTCG CCACGAACCT TGGCAGCCCA 480

TTCTTCGATG ACCGAGTCAT AGGCAACAAC ACCAGCGATC GAACCCCAGC CGCCAGCATA 540

CTTCAGGCCG CCAACAACGT CAGGCATGTA GCCGTCGATG CGGTAGTTGG TCGTGCCAGT 600

GTAACCACCG TCGTTGTCGC CACCCTGTTC GAGAGCGATC ACAGCCGAGA AGCCGTTTCC 660

GCCAGTGAAG GTGTACGAGA TCTTGCCGGT GCGGTAGGAG CCAGCCGAGA TCACGTCATC 720

GTTGATGACA TCGCCGAGGT AACCGGTGAA GGTATGGAAT TCCGATTCAT CGATACCAAC 780

GCGCAGACCA CCGAGCTGGA TATACGCGAA CTCCATGACG GTGCCGCTGC TGGTTTCATT 840

ACCATATTTA CCATCTACGC CCGAATTGTT CGCAGCATAG TTGAAGCGCA GTTCGGTGAA 900

GGTCTTGAGG GTGCCGAGTT CGGTTTCCGA ACCGGTGGAA ACGCGGAGTG CGAAACGAGC 960

GCTCTTGTCC CAGCCATTGC GGTCGGTACC GGAGTAAACG TCATCGCCGC CCTTTACGTC 1020

GTAACGGACG TAACCATGGA CGCGCAGGCA GGTTTCGGTG CCCGGAATGT AGAAGTAGCC 1080

AGCGCCGTAA GCGTCGCAAA CGCGGACATA TTCAACGGCT TCGGGCTCTG GCGCGACGAT 1140

TGCGTCGGCA GCCTGAGCGC CGGAAGCTGC AACCAGAGCT GCAGCGGAGC CAAGGAGAAG 1200

-continued

```
GCTCTTGATG TTCATTTCTG ACCTCCAGTC AAAGTTAAAA ATGGGTCTGG GCATTCTGAT   1260
TTGGCTGAAG GACAACCTGT CCCCATCCCC TAATTGAAAA AGTCGCCCCG AAGCGCTCCT   1320
TCTTCTGAAA GTGAAGATAC TCGCCCATTT ATTCGTTTCA ACATCGAATA TGTTCTCACA   1380
ACCTTTACGG TGCTGCTATG AAGGGCAGTT GTTGCTGAAA TGACACGAAA TTACCTGCTT   1440
TAGCTCGGCG GATTCATGCT TTATTAACAT AAGTGAACGC GAATTAACCG ATGTTAACGT   1500
TTGAAAATGC AAGTTTTTTA GGATCGCCTA CCGAATAAAG CCGCGAATCT TTCGTCGAAA   1560
CAGCCCTTAA CGGAATATGT CGGCAAGGTG GCAAGAATCG TCTGAACGGA GAGCAGAAAC   1620
CTCGAATCCG TTTCATTTAA TAAGGGCAAG TGCGTGCCGG TGCTAAATTG TGGGCCTTTT   1680
TAAGCGCGCT ATATATATAA AGAGAATAAT CCGCAGGAAA TTTTACCAGT TAATGCGTAA   1740
ATCGCTTGAA ATGCCCAGGC GTACCGGTTA TCTCGCCTTT ACCGGAGAGG TGGCCGAGTG   1800
GTCGAAGGCG CTCCCCTGCT AAGGGAGTAG ACCTCAAAAG GGTCTCGTGG GTTCGAATCC   1860
CATCCTCTCC GCCAGTTTTT CCAATATCCC AGCAAATCTT TATGTGTTCG ACGCGCTTGA   1920
TTTCATACGG AATCGGCTTT TACCCCTCGC GCACTGAATC TCTGTTTTTC CAGGCTACGA   1980
ATCCAGAAAA CAAGCAAGCC ATTGATAAGT AATGGCTATT CAAAATTCTG GCGATTCTTG   2040
ACTGGAGGTC AGAAATGAAC ATCAAGAGCC TTCTCCTTGG CTCCGCTGCA GCTCTGGTTG   2100
CAGCTTCCGG CGCTCAGGCT GCCGACGCAA TCGTCGCGCC AGAGCCCGAA GCCGTTGAAT   2160
ATGTCCGCGT TTGCGACGCT TACGGCGCTG GCTACTTCTA CATTCCGGGC ACCGAAACCT   2220
GCCTGCGCGT CCATGGTTAC GTCCGTTACG ACGTAAAGGG CGGCGACGAC GTTTATACCG   2280
GCTCGGATCG TAAAGGCTGG GACAAGGGCG CTCGTTTCGC ACTCATGTTC AACACGAATT   2340
CGGAAACCGA ACTCGGCACA CTCGGCACCT ATACTCAGCT GCGCTTCAAC TACACCAGCA   2400
ACAATTCACG TCATGATGGC CAATACGGCG ATTTCAGCGA TGATCGTGAT GTCGCTGATG   2460
GCGGCGTAAG CACCGGCACC GATCTGCAGT TTGCATATAT CACGCTTGGT GGTTTCAAGG   2520
TTGGTATCGA CGAATCCGAA TTCCATACCT TCACCGGTTA CCTCGGTGAT GTCATCAACG   2580
ATGATGTCGT CGCTGCTGGC TCCTACCGCA CCGGCAAGAT CGCCTACACC TTCACCGGCG   2640
GAAACGGCTT CTCGGCTGTG ATCGCTCTCG AACAGGGTGG CGAAGACGTT GACAACGATT   2700
ACACGATCGA CGGTTACATG CCGCACGTTG TTGGCGGCCT GAAATATGCT GGCGGCTGGG   2760
GTTCGATCGC TGGTGCTGTT GCCTATGACC CGGTCATCGA AGAATGGGCT ACAAAGGTTC   2820
GTGGCGACGT CAACATCACC GACCGGTTCT CGGTATGGCT GCAGGGCGCA TATTCGTCCG   2880
CAGCGACGCC GAACCAGAAC TACGGTCAGT GGGGCGGCGA TTGGGCTGTC TGGGGTGGTG   2940
CAAAGTTCAT TGCCACCGAA AAGGTAACCT TCAATCTGCA GGCTGCGCAT GACGACTGGG   3000
GCAAGACCGC AGTTACCGCC AACGTCGCTT ATCAGCTCGT TCCCGGATTC ACCATTACGC   3060
CGGAAGTTTC CTACACCAAA TTTGGTGGCG AGTGGAAAGA CACCGTTGCT GAAGACAATG   3120
CCTGGGGCGG TATCGTTCGC TTCCAGCGCT CGTTCTAATC AGATCGACGT TAAGCATAGG   3180
GCGCCAACGG TTTCCCGTTG GCGCCGGTTC ATTTGAAACA GCGTTCACGA AAGCGTGAGA   3240
ATCGATTCTT CCGGAATGGG GATTCCAGGC GGATCGACAA TTGAGGGAAT TGCGGGGACG   3300
ACAAAAAGCT GGGGGCAACC GGGGGGTCTT GTAAAGGATT GAGCCAC                  3347
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

-continued

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGCGAACTCC ATGACGGTGC CGC 23

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCTTGGCTCC GCTGCAGCTC TGGT 24

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CAGGCGATCT TCCGCGACCC C 21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGGGATGGGG ACAGGTTGTC C 21

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGGGTCTGGG CATTCTGATT TGGCTG 26

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCGCCAGAAT TTTGAATAGC CATTAC 26

(2) INFORMATION FOR SEQ ID NO:15:

-continued

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCTTGGCTCC GCTGCAGCTC TGGT 24

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGTTGTCAAC GTCTTCGCCA CCC 23

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCGGCGGCCA ACGGGAAACC G 21

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGGCTTTACC CCTCGCGCAC 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TGGCTCAATC CTTTACAA 18

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-continued

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TCGTGATGTC GCTGATGG 18

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATGTCGTCGC TGCTGGCTCC 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ATGTCGTCGC TGATGGCTCC 20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ACGTGATCTC GGCTGGCTCC 20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TGTTGTTGCC TATGACTCGG 20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TGCTGTTGCC TATGACCCGG 20

-continued

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCCCGAAAAG GCAACCTTCA 20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CACCGAAAAG GCAACCTTCA 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CACCGAAAAG GTAACCTTCA 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AGACCGCAGT TACCGCCAAC 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AGACGGCAGT TACGGCTAAC 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-continued

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GTCGCTTATC AGCTCGTTCC 20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GTTGCTTACG AACTGGTTCC 20

We claim:

1. A method for identifying a species or biovar of Brucella comprising the steps of:

releasing DNA from a test sample;

amplifying a gene sequence of a Brucella omp2 gene locus as defined in FIGS. 2A–O from the released DNA, said gene sequence having sufficient diversity to distinguish species of Brucella;

analyzing the hybridization of the amplified gene sequence with a panel of DNA probes to identify a species or biovar of Brucella.

2. The method of claim **1** wherein said amplified gene sequence is a sequence between nucleotides 2470 and 3346 of the Brucella omp2 gene consensus sequence.

3. The method of claim **1** wherein said amplifying is by polymerase chain reaction.

4. The method of claim **3**, wherein the polymerase chain reaction is primed with an oligonucleotide pair which anneals to the omp2 gene locus of Brucella to amplify a sequence between nucleotides 2470 and 3346 of the Brucella omp2 consensus sequence.

5. The method of claim **3**, wherein the polymerase chain reaction is primed with an oligonucleotide pair selected from those having Seq. Id. Nos. 9–20.

6. The method of claim **4**, wherein said oligonucleotide pair is that having Seq. Id. Nos. 19 and 20.

7. The method of claim **6**, wherein said panel of DNA probes includes one or more of the probes having Seq. Id. Nos. 21–32.

8. The method of claim **7**, wherein said panel includes at least two probes having Seq. Id. Nos. 21–32.

9. The method of claim **1**, wherein said test sample is animal fluid or tissue.

10. The method of claim **7** wherein said test sample is urine, blood, milk, semen, vaginal or rectal secretions.

11. The method of claim **8**, wherein said test sample is milk.

12. The method of claim **1**, wherein said analyzing is by dot blot, DNA hybridization, or by agarose gel electrophoresis.

13. The method of claim **12**, wherein said analyzing is using radiolabeled oligonucleotide probes.

* * * * *