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OmpA and antigenic diversity of bovine *Chlamydophila pecorum* strains

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ABSTRACT

Infections with the intracellular bacterium *Chlamydophila (C.) pecorum* are highly prevalent worldwide in cattle. These infections cause significant diseases such as polyarthritis, pneumonia, enteritis, genital infections and fertility disorders, and occasionally sporadic bovine encephalomyelitis. Subclinical respiratory infections of calves with *C. pecorum* have been associated with airway obstruction, pulmonary inflammation, and reduced weight gains. This investigation examined four chlamydial strains with biological properties of *C. pecorum* isolated from feces of clinically normal cattle, from calves with pneumonia, and from bulls with posthitis. The objective was to characterize the evolutionary relationships of these bovine chlamydial isolates to other chlamydiae by genetic analysis of the *ompA* gene, and by the immunological cross-reactivities in Western immunoblot analysis. PCR typing of the *ompA* gene identified these isolates as *C. pecorum*. The OmpA-deduced amino acid dissimilarities between these four strains spanned 10–20%. In phylogenetic analysis, the four isolates clustered with *C. pecorum* ruminant, porcine, and koala strains of different geographic origins rather than with each other. All four isolates showed different patterns of Western immunoblot reactivity with antiserum against bovine *C. pecorum* strain LW63, and, interestingly, no cross-reactivity of the OmpA proteins with the anti-LW613 OmpA antibodies. These data underscore the polyphyletic population structure of *C. pecorum* and suggest that the spectrum of *C. pecorum* OmpA proteins in a host species can occupy the entire evolutionary bandwidth within *C. pecorum*. The variant immunoblot reactivities support the notion of considerable genomic plasticity of *C. pecorum*.

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1. Introduction

Intracellular bacteria of the family *Chlamydiaceae* are some of the most prevalent microorganisms in the biosphere, infecting virtually all eukaryotic cells (Everett et al., 1999). These bacteria also occur widely in livestock (Longbottom, 2004), and ruminants harbor largely two chlamydial species, *Chlamydophila (C.) abortus* and *Chlamydophila pecorum* (Fukushi and Hirai, 1992). While research efforts have focused for a long time on *C. abortus*, *C. pecorum* has attracted more attention over the last decade, because it is more widespread in cattle than *C. abortus* (Jee et al., 2004) and causes numerous significant disease manifestations, such as sporadic bovine encephalomyelitis (McNutt and Waller, 1940), polyarthritis (Storz et al., 1966), pneumonia (Wilson and Thomson, 1968), enteritis (Doughri et al., 1974), as well as vaginitis and endometritis (Wittenbrink et al., 1993). In koalas, *C. pecorum* is the most prevalent cause

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of conjunctivitis and severe urogenital infections leading to infertility (Jackson et al., 1999). In swine, *C. pecorum* infection has been associated with pneumonia, polyarthritis, and abortion (Koelbl, 1969). Aside from severe disease, essentially ubiquitous chronic low-level respiratory *C. pecorum* infections in calves have also been associated with subclinical, but significant airway obstruction, pulmonary inflammation, and reduced weight gains (Jaeger et al., 2007; Reinhold et al., in press). These facts suggest that *C. pecorum* infections potentially create substantial losses in the livestock industries, and efforts at better understanding, mitigating, and preventing these infections should be increased.

Wittenbrink et al. (1988, 1990) isolated in Germany a number of chlamydial strains from the feces of clinically normal cattle, from calves with pneumonia, and from heifers and bulls with endometritis and fertility disorders. The biological properties of these isolates resembled *C. pecorum*, and the objective of this investigation was to speciate several of these isolates and further characterize their evolutionary relationships to other chlamydiae.

2. Materials and methods

2.1. Chlamydial isolates

Four bovine chlamydial strains, B6, B13, Bahr14, and Bahr23, were specifically studied in this investigation (Table 1). These chlamydiae had been isolated by chicken embryo or BGM cell culture at the Veterinary Medical University in Hannover, Germany (Wittenbrink et al., 1988; Wittenbrink, 1990; Wittenbrink, unpublished data). The chlamydial strains were maintained by serial passage in Buffalo Green Monkey Kidney (BGMK) cells, and purified elementary bodies (EBs) were prepared from supernatants of infected cultures by centrifugation on a step-gradient of 30% RenoCal-76 and 50% sucrose (Li et al., 2005).

2.2. Typing of the *ompA* locus

DNA was extracted as described from purified EBs (Kaltenboeck et al., 1997). A fragment of the *ompA* gene, i.e. the chlamydial major outer membrane protein gene, was first amplified using two sets of genus-specific primers in a nested PCR (Kaltenboeck et al., 1997). For determination of the chlamydial species and alleles, the amplification product of the second PCR was first subjected to hemi-nested species-specific PCRs, followed by the appropriate hemi-nested allele-specific PCRs as described by Kaltenboeck et al. (1997). All products were analyzed by 2% agarose gel electrophoresis and visualized by staining with ethidium bromide.

2.3. Phylogenetic analysis

For precise determination of the *C. pecorum* alleles of isolates B6, B13, Bahr14, and Bahr23, the DNA sequence of the main portion of the *ompA* gene including all four variable domains was determined. The *ompA* gene was amplified with *Chlamydiaceae* family-specific primers 9CHOMP and CHOMP371 (Kaltenboeck et al., 1993). The amplified fragment was purified by 1.5% agarose gel electrophoresis and extraction with binding to and elution from a glass fiber matrix. Nucleotide sequences of both strands were determined by automated DNA sequencing using the PCR primers and internal genus-specific primers as described by Kaltenboeck et al. (1993).

For species identification, the partial *ompA* sequences of isolates B6, B13, Bahr14, and Bahr23 were first aligned with the prototype sequences of the nine *Chlamydiaceae* species (Kaltenboeck et al., 1993; Everett et al., 1999). Subsequent analyses were performed with the six other *C. pecorum ompA* genes available on GenBank (Table 1). Because of the higher biological significance of peptide versus nucleotide sequences, the *C. pecorum* sequences were aligned as peptides using the Clustal W multiple alignment algorithm (Thompson et al., 1994) as implemented in the Vector NTI software package (Invitrogen, Carlsbad, CA). The Blosum residue substitution matrix was used to weigh the amino acid substitutions in the sequence alignment algorithm. The OmpA protein distance matrix derived from this alignment was used to calculate a best-fit phylogenetic tree by the neighbor-joining algorithm of Saitou and Nei (1987).

Jackson et al. (1997) published the variable domain IV (VD IV) sequence of 15 *C. pecorum* strains isolated from koalas, and of one strain isolated from bovine abortion. To evaluate the relative phylogenetic position of these strains, the VD IV peptide sequences of the five koala *C. pecorum ompA* genotypes and of the bovine isolate along with the VD IV sequences of the strains shown in Table 1 were aligned, and a phylogenetic tree was derived by neighbor-joining as described above.

Table 1
Characteristics of 6 bovine, 1 ovine, 1 caprine, and 2 porcine *C. pecorum* isolates analyzed or used as references

C. pecorum isolate	Host	Clinical condition	Country of origin	Reference	GenBank accession
66P130	*Bos taurus*	Normal feces	USA	Kaltenboeck et al. (1993)	M73034
B13	*Bos taurus*	Posthitis	Germany	Wittenbrink et al., 1990	EU350136
Bahr14	*Bos taurus*	Pneumonia	Germany	Wittenbrink, unpublished data	EU350137
1710S	*Sus scrofa*	Abortion	Austria	Kaltenboeck et al. (1993)	M73033
B6	*Bos taurus*	Normal feces	Germany	Wittenbrink et al. (1988)	EU350135
P787	*Ovies aries*	Polyarthritis	United Kingdom	Anderson et al. (1996)	Z18756
Bahr23	*Bos taurus*	Pneumonia	Germany	Wittenbrink, unpublished data	EU350138
LW613	*Bos taurus*	Polyarthritis	USA	Kaltenboeck et al. (1993)	M73042
CapraNZ	*Capra hircus*	Unknown	New Zealand	O'Keefe et al. (2001)	AY055109
L71	*Sus scrofa*	Polyarthritis	Austria	Kaltenboeck et al. (1993)	M73039

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2.4. Immunoblot analysis of bovine chlamydial isolates

For further evaluation of the polymorphisms of the *C. pecorum* alleles of isolates B6, B13, Bahr14, and Bahr 23, EB proteins were analyzed by immunoblot reactivity to an antiserum against *C. pecorum* LW613. Five microgram protein aliquots of density-gradient purified EBs were solubilized at room temperature in Laemmli sample buffer and resolved by reducing discontinuous SDS-12% polyacrylamide gel electrophoresis (PAGE) (Laemmli, 1970). After separation, proteins were transferred to Immobilon-P membrane (Millipore, Bedford, MA) by the semi-dry technique of Towbin et al. (1979).

Antibodies against *C. pecorum* LW613 were raised by two subcutaneous and intravenous priming and two booster immunizations of rabbits with 106 egg infectious doses of viable yolk-sac propagated, gradient-purified EBs. Membranes were blocked with 3% ovalbumin, incubated with 1:100 diluted rabbit hyperimmune serum, and immunoreactive chlamydial proteins were detected by affinity-purified alkaline phosphatase-conjugated swine anti-rabbit IgG (H + L) and NBT/BCIP (Sigma, St. Louis, MO).

3. Results

3.1. Typing of the **ompA** locus

All four bovine chlamydial isolates analyzed in this study, B6, B13, Bahr14, and Bahr23, yielded strong, specific bands in the primary and secondary *Chlamydiaceae* family-specific *ompA* PCRs and in the *C. pecorum* species-specific PCR, but not in the *C. psittaci, C. pneumoniae*, or *Chlamydia* trachomatis species PCRs (Kaltenboeck et al., 1997). This result established unequivocally that all four bovine chlamydial isolates belonged to the species *C. pecorum*. Of all *C. pecorum* allele-specific PCRs, only *C. pecorum* strains Bahr23 and B6 gave weak bands after 15 cycles in the LW613 PCR, but not Bahr14 or B13 (data not shown). None of the four isolates showed an amplification fragment in the 66P130, 1710S, or L71 *C. pecorum* allele-specific PCRs (Kaltenboeck et al., 1997). These data suggested that the four bovine *C. pecorum* isolates represented novel *ompA* alleles.

3.2. Phylogenetic analysis

To obtain an unambiguous answer about the *ompA* type of the four bovine *C. pecorum* isolates, the nucleotide sequence of amplification products of genus-specific *ompA* PCRs was determined. These PCRs used primers close to the 3′- and 5′-ends of the *ompA* open reading frame (ORF), and the product allowed sequencing of ~70% of the *ompA* coding region including all four variable domains (Kaltenboeck et al., 1993). Nucleotide as well as translated peptide sequences were aligned with prototypes of all chlamydial species (data not shown), and clustered with the *ompA* sequence of *C. pecorum* LW613, which is identical to the *C. pecorum* type strain E58 (Kaltenboeck et al., 1993). These data confirmed the species of the four bovine isolates as *C. pecorum*, and further phylogenetic analyses were performed only with all published *C. pecorum* sequences.

Because of the biological significance, only the translated *ompA* peptide sequences were aligned (Fig. 1). Each of the four bovine isolates has a unique OmpA sequence and represents a novel *ompA* allele of *C. pecorum*. The partial OmpA amino acid dissimilarities between these four strains span 10–20%, covering the complete range of distances within all *C. pecorum* OmpA sequences (Table 2). Almost all amino acid polymorphisms are clustered within the OmpA variable domains. The range of partial OmpA distances within *C. pecorum* covers approximately two thirds of the maximum OmpA distance within all nine *Chlamydiaceae* species, and is similar to the OmpA distances within human serovars of *C. trachomatis* (Kaltenboeck et al., 1993).

An unrooted neighbor-joining phylogenetic *C. pecorum* species tree of the OmpA proteins was constructed from the dissimilarity matrix of all *C. pecorum* partial OmpA proteins available in GenBank (Fig. 2). They separate into three main clades that originate from the base of the tree. Two of the bovine *C. pecorum* strains each sequenced in this study segregate to the two larger clades of ruminant and porcine isolates, comprised of three and five strains, respectively. The third and smallest clade is comprised of a caprine *C. pecorum* strain from New Zealand and porcine strain L71 from Austria. These data underscore the polyphyletic population structure of *C. pecorum* and suggest that the spectrum of *C. pecorum* OmpA proteins in a host species can occupy the entire evolutionary bandwidth allowed by the functional constraints on the chlamydial major outer membrane protein, and that similarities between *C. pecorum* strains isolated from different host species or continents may be greater than similarities between isolates from the same host species or area.

Table 2
Amino acid dissimilarities (distances) among the aligned published partial *ompA* sequences of the 8 ruminant and 2 porcine *C. pecorum* isolates

	66P130	B13	Bahr14	1710S	B6	P787	Bahr23	LW613	CapraNZ	L71
66P130	–	12	16	16	19	18	17	18	17	16
B13		–	17	19	20	19	19	20	18	19
Bahr14			–	12	16	16	16	14	10	10
1710S				–	13	14	13	11	10	10
B6					–	11	10	14	14	15
P787						–	13	12	15	15
Bahr23							–	12	13	13
LW613								–	9	9
CapraNZ									–	4
L71										–

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           1                          variable  domain I   48
66P130     LRVGFYGDYVFDRVLKTDVPKMFPMGAAPTGSS---TVSPNQAQ-ERD
B13        ····· ····· ····· ····· ····QK·S···T···AG---PLN··S·I-···
Bahr14     ····· ····· ····· ····· ····· ····S··T···S·G---AAATSNTKTD·E
1710S      ····· ····· ····· ····· ····· ····S·····SPD---NPADSTVKQ··Q
B6         ····· ····· ····· ····· ····QK·S··PI·VSN·---AAADSAIPT···
P787       ····· ····· ····· ····· ····QK·S··PI··S·T---SLEDSAILT··N
Bahr23     ····· ····· ····· ····· ····S·Q·S·····SAANPSSPADSVSKAD·P
LW613      ····· ····· ····· ····· ····S···L··T···SPN---NAADSSTTA··A
CapraNZ    ····· ····· ····· ····· ····S···L··T···SP·---SAADSNTTA··A
L71        ····· ····· ····· ····· ····S···L··T···SPN---SAADSNTTA··A

           49                                                 96
66P130     NPAYNTHMHDAEWFTNAGYIALNIWDRFDIFCTLGATSGYFKGNSKSF
B13        ····KK··Q····· ····· ····· ····· ····· ····N·····T··
Bahr14     ····GK····· ····· ····· ····· ····· ····· ····· ····S··
1710S      ····GK····· ····· ····· ····· ····· ····· ····· ····S··
B6         ·A··GK····· ····· ····· ····· ····· ····· ····· ····S··
P787       ·AG·GK·····L····· ····· ····· ····· ····· ····· ····S··
Bahr23     ····GK····· ····· ····· ····· ····S····· ····· ····A··
LW613      ····GK····· ····· ····· ····· ····V····· ····· ····S··
CapraNZ    ····GK····· ····· ····· ····· ····· ····· ····· ····A··
L71        ····GK····· ····· ····· ····· ····· ····· ····· ····A··

           97     variable domain II                          144
66P130     NLIGLIGLAGSTLNNTFYPNASISNGVVELYTDTTFSWSVGARGALWE
B13        ·····VQ·T··AS-M····· ····· ····· ····· ····· ····· ····
Bahr14     ·····S····TS-M····· ····· ····· ····· ····· ····· ····
1710S      ·····IS·····D-KL···N····· ····· ····· ····· ····· ····
B6         ·····IS·A··DD-KL···N····· ····· ····· ····I····· ····
P787       ·····IS·AD··S-NV···N····· ····· ····· ····· ····· ····
Bahr23     ·····IS·TD···-KL····· ····· ····· ····· ····· ····· ····
LW613      ·····IS··S·EG-K····N····· ····· ····· ····· ····· ····
CapraNZ    ·····IS·T··DQ-K····· ····· ····· ····· ····· ····· ····
L71        ·····IS····DQ-K····· ····· ····· ····· ····· ····· ····

           145                                          variable
66P130     CGCATLGAEFQYAQSKPRVQELNVLSNVAQFTVHRPKGYVNQPLPLPI
B13        ····· ····· ····· ····· ····I····· ····· ····Q·····A····L
Bahr14     ····· ····· ····· ····· ····· ····· ····K·Q··IG·S····E
1710S      ····· ····· ····· ····· ····· ····· ····K·Q··IG·Q····T
B6         ····· ····· ····· ····· ····· ····· ····K·····K·····
P787       ····· ····· ····· ····· ····· ····· ····K·R···G·····L
Bahr23     ····· ····· ····· ····· ····· ····· ····K·····E·····
LW613      ····· ····· ····· ····· ····· ····· ····K·R···G·T····L
CapraNZ    ····· ····· ····· ····· ····· ····· ····I·K·Q···G·S····T
L71        ····· ····· ····· ····· ····· ····· ····K·Q···G·S····T

           domain III                                          240
66P130     TAGTSTDTATNLKNATINYHEWQVGAALSYRLNMLVPYIGVQWSRATF
B13        E···-··A····· ····· ····· ····· ····· ····I····· ····· ····
Bahr14     N····AA·D--····· ····· ····· ····· ····I····· ····· ····
1710S      ·····NA·D--····· ····· ····· ····· ····· ····· ····· ····
B6         S···AA·SNEK····· ····· ····· ····· ····I····· ····· ····S·
P787       ····A··SNDK····· ····· ····· ····· ····· ····· ····· ····
Bahr23     S···A··SNEK····· ····· ····· ····· ····I····· ····· ····S·
LW613      S···E··SSDK····· ····· ····· ····· ····· ····· ····· ····
CapraNZ    N····NA·D--····· ····· ····· ····· ····I····· ····· ····
L71        N····NASD--····· ····· ····· ····· ····I····· ····· ····

           241                 variable domain IV          286
66P130     DADTIQIAQPKLATAVLNLTTWNPTLLGEITSQDLDTANKYADTLQ
B13        ····· ····· ····· ····· ····· ····TN·····FS····
Bahr14     ····· ····· ····SPIFY····· ····· ····R··GTTL--D····S··
1710S      ····· ····· ····SPIFR····· ····· ····QA·QV·GT---I···A··
B6         ····· ····· ····SPIF····· ····· ····QAI·V·GV----F··A··
P787       ····· ····· ····E····SPIF····· ····· ····QA··V·SG----F··S··
Bahr23     ····· ····· ····E····SPIF····· ····· ····QA··V·GV----F··A··
LW613      ····· ····· ····E····SPIF····· ····· ····QA·TV·GT----F··S··
CapraNZ    ····· ····· ····SPIF····· ····· ····QA·TVPS---D····S··
L71        ····· ····· ····V·····SPIF····· ····· ····QA··VGS---D·····
```

Fig. 1. Alignment of the deduced partial *C. pecorum* OmpA protein sequences. Translated nucleotide sequences were aligned by the Clustal W algorithm and arranged in the order of phylogenetic relatedness. Residues identical to 66P130 are shown as dots, and sequence gaps introduced during alignment are shown as hyphens. Amino acid 1 in the alignment corresponds to amino acid 60 of *C. psittaci* MN OmpA, and amino acid 286 to MN amino acid 350 (Kaltenboeck et al., 1993).

To further examine this hypothesis, the phylogenetic relationships among peptide sequences of the OmpA VD IV of the above strains, of the five koala *C. pecorum* A–E *ompA* genotypes and a novel Australian bovine abortion isolate B1 (Jackson et al., 1997) were reconstructed. The resultant phylogenetic tree (Fig. 3) shows the same three clades as observed in Fig. 2, but with slightly variant branching order, presumably due to the lower resolution obtained by use of the short VD IV sequences. In confirmation of the polyphyletic population structure of *C. pecorum*, each of the koala *ompA* genotypes clustered separately with ruminant or porcine genotypes rather than with the other koala genotypes. Koala *ompA* genotype C formed a separate branch in the *C. pecorum* OmpA VD IV phylogenetic tree, and koala genotype D was identical to bovine genotype LW613. Interestingly, the Australian bovine abortion isolate B1 was identical to the German bovine pneumonia isolate Bahr14 sequenced in this investigation.

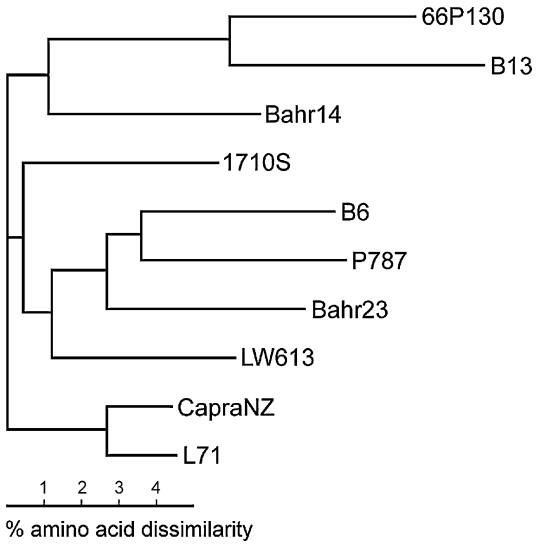

Fig. 2. Unrooted phylogenetic tree of the deduced partial *C. pecorum* OmpA proteins available in GenBank. The phylogenetic tree was constructed by the neighbor-joining algorithm from the dissimilarity matrix of the aligned sequences shown in Fig. 1 (positions 1–286).

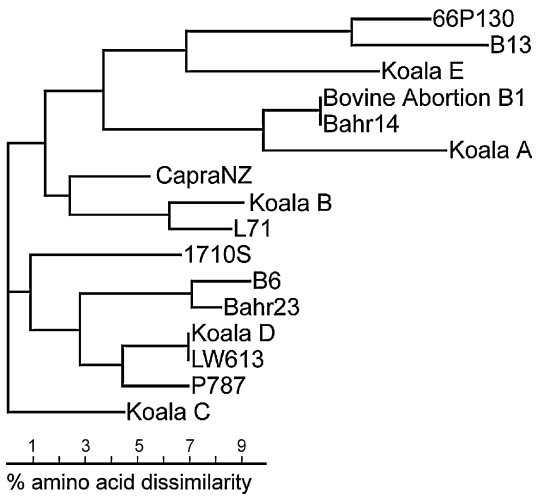

Fig. 3. Unrooted phylogenetic tree of all published *C. pecorum* OmpA variable domain IV peptide sequences (positions 226–286 in Fig. 1). The five koala OmpA VD IV genotypes A–E and bovine abortion B1 genotype were reported by Jackson et al. (1997).

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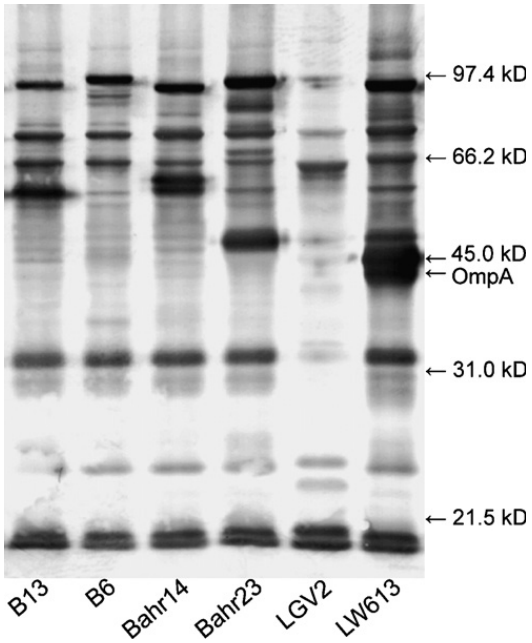

Fig. 4. Immunoblot of bovine *C. pecorum* strains analyzed in this study. Purified EBs were resolved by SDS-PAGE, transferred to a membrane, and reacted with rabbit anti-LW613. Reactivity was visualized by use of alkaline phosphatase-conjugated swine anti-rabbit IgG. The lymhogranula venereum 2 (LGV2) strain of *Chlamydia trachomatis* (isolate LGV-2 434/Bu) is used as a distantly related chlamydial control.

3.3. Immunoblot evaluation of the relatedness of bovine *C. pecorum* antigens

To obtain further information on the diversity of bovine *C. pecorum* strains, the relationship among immunogenic macromolecules was probed by Western immunoblot analysis using rabbit antisera raised against native *C. pecorum* LW613 EBs (Fig. 4). All four novel bovine *C. pecorum* isolates show different patterns of reactivity with the anti-LW613 antibodies, with conserved reactivities of antigens of ~18, 19, 33, 62, and 80 kD molecular weight, but succinct differences in antigens between 40 and 97 kD. These data further confirm the high diversity of the *C. pecorum* population. Most interestingly, none of the novel bovine isolates of *C. pecorum* shows significant binding of the anti-LW613 OmpA antibodies to the putative chlamydial OmpA protein at ~42 kD, while the LW613 strain binds strongly (Fig. 4). This indicates that the polymorphisms of the OmpA proteins within bovine *C. pecorum* are sufficient to abolish antibody cross-reactivity of the major outer membrane proteins.

4. Discussion

The objective of this investigation was to characterize the relationship of four bovine chlamydial isolates to other ruminant chlamydiae, with *C. abortus* and *C. pecorum* as the main chlamydial species found in cattle. Identification of these isolates as *C. pecorum* is consistent with findings that indicate a high prevalence of these agents in cattle (Jee et al., 2004; Reinhold et al., in press).

The results of this investigation are further consistent with data obtained by different methods that indicate significant heterogeneity among *C. pecorum* strains. Earlier studies by Anderson et al. (1996), Jackson et al. (1997) and Kaltenboeck et al. (1993) have revealed large polymorphisms of the *ompA* gene. This study confirms these polymorphisms, and the conserved polyphyletic structure of the *C. pecorum* OmpA relationships (Jackson et al., 1997; Kaltenboeck et al., 1993). In the partial OmpA phylogenetic tree (Fig. 2), comprised of ruminant and porcine *C. pecorum* strains, the novel German bovine isolates group with ruminant strains of different geographic origins rather than with each other, and the porcine strains isolated in Austria (Kaltenboeck et al., 1993) group with ruminant strains rather than with each other. Similarly, in the OmpA VD IV tree, the koala genotypes do not cluster, but are individually most closely related to ruminant and porcine strains of different geographic origins, and Australian Koala genotype D is actually identical to ruminant *C. pecorum* strain LW613, which was isolated from a calf with polyarthritis in the USA. These data suggest that *C. pecorum*, even if well adapted to a ruminant host, has not lost the ability to cross into new host species, and that multiple strains have done so successfully for the koala host (Jackson et al., 1997).

The existence and considerable genetic diversity of multiple *C. pecorum ompA* types in a particular host species, such as cattle or koala, is reminiscent of the *ompA* sequence diversity of human serovars of *C. trachomatis* (Kaltenboeck et al., 1993). Similar to *C. trachomatis* serovars, the OmpA proteins of the novel bovine isolates show little or no antibody cross-reactivity (Fig. 4). These data suggest selection of *C. pecorum* for avoidance of antibody binding to surface antigens at the preferred mucosal habitat of *C. pecorum* (Doughri et al., 1974; Jee et al., 2004).

Anderson et al. (1996) present species-supporting evidence for genomic stability of *C. pecorum* by probing of Southern blots of chlamydial genomic DNA with *C. pecorum* genomic and 16S rRNA, and by restriction analysis of the 16S rRNA and *ompB* genes. However, Fukushi and Hirai (1989), Salinas et al. (1996), and Liu et al. (2007) showed also strong evidence for *C. pecorum* genomic plasticity. Fukushi and Hirai separated ruminant *C. pecorum* strains into two RFLP types by restriction analysis of genomic DNA. Using six *C. pecorum*-reactive monoclonal antibodies, Salinas et al. (1996) established 16 different reactivity patterns of these mAbs with a panel of 18 ruminant *C. pecorum* strains. Liu et al. (2007) sequenced the *rrn-nqrF* intergenic segment of 10 *C. pecorum* isolates of ruminant and porcine origin. They found that all *C. pecorum* strains showed a specific type of the *rrn-nqrF* intergenic segment, but that each strain harbored a different number of octamer repeats in this segment. In the present study, the highly variant Western immunoblot reactivities of the four novel bovine isolates with LW613 antiserum support the notion of considerable genomic plasticity of *C. pecorum*. In addition, the antibody reactivities suggest that selection for immune evasion is also operative at other loci encoding *C. pecorum* surface proteins.

A key question is if these data truly indicate stable genomic differences between *C. pecorum* strains that are consistently associated with the same *ompA* genotype, or if they indicate minor random polymorphisms. In other words, is only a small number of immune-exposed

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proteins in an otherwise stable genome hypervariable due to selection for immune evasion, as in *C. trachomatis*, or do the different *ompA* types reflect truly divergent genomes of *C. pecorum*? An answer to this question may be obtained by sequencing a multiplicity of loci using the emerging genome sequence of *C. pecorum* type strain E58 (Myers, Kaltenboeck, et al., unpublished data) for design of PCR primers. Given the high worldwide prevalence of *C. pecorum*, and the propensity for subclinical losses and clinically manifest diseases resulting from these infections, vaccine protection against diseases induced by *C. pecorum* is desirable. Further understanding of the genomic diversity of *C. pecorum* is important in this quest for a *C. pecorum* vaccine.

Conflict of interest statement

None declared.

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