Identification of Immunologically Relevant Proteins of *Chlamydia abortus* Using Sera from Experimentally Infected Pregnant Ewes†

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*Chlamydia abortus* is an intracellular pathogen and the etiological agent of enzootic abortion of ewes (EAE). *C. abortus* has a biphasic development cycle; extracellular infectious elementary bodies (EB) attach and penetrate host cells, where they give rise to intracellular, metabolically active reticulate bodies (RB). RB divide by binary fission and subsequently mature to EB, which, on rupture of infected cells, are released to infect new host cells. Pregnant ewes were challenged with 2 × 10⁶ inclusion forming units (IFU) of *C. abortus* cultured in yolk sac (comprising both EB and RB). Serum samples were collected at 0, 7, 14, 21, 27, 30, 35, 40, and 43 days postinfection (dpi) and used to identify antigens of *C. abortus* expressed during disease. Additionally, sera from fetal lambs were collected at 30, 35, 40, and 43 dpi. All serum samples collected from experimentally infected pregnant ewes reacted specifically with several antigens of EB as determined by one-dimensional (1-D) and 2-D gel electrophoresis; reactive antigens identified by mass spectrometry included the major outer membrane protein (MOMP), polymorphic outer membrane protein (POMP), and macrophage infectivity potentiator (MIP) lipoprotein.

*Chlamydia abortus*, an obligate intracellular Gram-negative bacterium, causes enzootic abortion of ewes (EAE), an important disease of sheep (4–6, 28, 36). In the United Kingdom, *C. abortus* was responsible for 37% of ovine abortions diagnosed between 2000 and 2006 (28). In Scotland, *C. abortus* was determined as the etiological agent for 32% of submitted cases in which a diagnosis was reached in 2009 (36). In Ireland, in 2008, 10.3% of submitted samples from ovine abortions were positive for *C. abortus* (6). However, reported figures are likely to be an underestimate, as the optimal sample for diagnosis of EAE is fresh ovine placenta, which is often omitted when samples are submitted for diagnosis.

*Chlamydia abortus* has a biphasic development cycle. Infectious extracellular elementary bodies (EB) are metabolically inactive and resistant to adverse environmental conditions. EB attach to host cells, invade, and develop into reticulate bodies (RB), which are metabolically active and multiply by binary fission within a specialized intracellular compartment known as an inclusion. Over a period of 48 to 72 h, inclusions grow in size, and RB mature into EB. The expanding inclusion subsequently ruptures the host cell membrane, thus releasing infectious EB to infect other host cells (47).

The complete genome sequence of *C. abortus* S26/3 has been reported (43). Two major antigens have been studied extensively to date: the major outer membrane protein (MOMP) of approximately 39 kDa (12, 14, 53) and the polymorphic outer membrane proteins (POMP), which are expressed with apparent molecular masses of 85, 90, and 105 kDa (12, 19–21, 40, 46, 47, 49). Other antigenic proteins identified using serum from an infected mouse include the small and large cysteine-rich proteins of 12 and 60 kDa, respectively (49), and a 27-kDa antigen that comigrates with a *Chlamydia trachomatis* macrophage infectivity potentiator (MIP)-like protein (23). The first *C. abortus* inclusion membrane protein, Inc766, was recently described (48).

This study used experimental infection of pregnant ewes with *C. abortus* to emulate naturally occurring disease processes. Purified EB were separated by one-dimensional (1-D) and 2-D gel electrophoresis and screened for reactivity with ovine sera by immunoblotting to identify antigens expressed during enzootic abortion of ewes.

**MATERIALS AND METHODS**

Culture and purification of RB and EB. An isolate of *Chlamyphyila abortus* (26) was inoculated into the yolk sac of a 7-day-old embryonated hen egg (18). After 7 days, *C. abortus* was harvested as previously described and stored at.
One milliliter of minimum essential medium supplemented with 1% nonessential amino acids, 0.1 mg/ml L-phenylalanine, 0.1 mg/ml Streptomycin sulphate, 5% fetal bovine serum (FBS) (Invitrogen Corporation), and 1 μg/ml cycloheximide (Sigma-Aldrich Ltd.) was added, and the cultures were incubated at 37°C in 5% CO₂ for 48 h (34). Cells were then fixed with acetic acid for 10 min, washed with phosphate buffered saline (PBS), and incubated with hyperimmunun anti-C. abortus sheep serum (1:400 dilution) or PBS (negative controls) for 1 h at 37°C. Cells were washed again with PBS and incubated with donkey anti-sheep IgG fluorescein isothiocyanate (FITC) conjugate antibody (Sigma-Aldrich Ireland Ltd.) for 1 h at 37°C. IFU were visualized and counted using a Nikon Eclipse E400 fluorescence microscope (Nikon UK Ltd.).

McCoy cells were inoculated with C. abortus-infected yolk sac as previously described, with slight modification (15): 25-cm² T flasks (Sarstedt Ltd., Ireland) were used and were centrifuged at 2,000 × g for 20 min. At 24 and 72 h postinoculation (hpi), cells were harvested using glass beads or cell scrapers. The 24-hpi cells (RB) were homogenized, the 72-hpi cells (EB) were sonicated, and each preparation was centrifuged (300 × g for 15 min) to remove gross cellular debris. Samples were then centrifuged over a 30% Urografin gradient (Urografin 370; Schering, Germany) at 50,000 × g for 45 min, at 10°C, as previously described (27). Pellets were pooled and purified further as previously described, with slight modifications (51). In brief, discontinuous Urografin gradients of 40, 35, and 30% (for RB) and 45, 40, and 35% (for EB) were used, and centrifugation was performed at 50,000 × g for 2 h at 10°C. After centrifugation, RB was situated in the 35% Urografin layer, or EB, situated between the 45 and 40% Urografin layers, were collected by piercing the tube with a 26-gauge three-eighths-inch needle on a 1-ml syringe (BD Plastipak). Samples were washed twice with Tris-KCl buffer and stored in sucrose phosphate glutamic acid buffer (150 μl/g liter sucrose, 7 mM KH₂PO₄, 14 mM K₂HPO₄, 1 g/liter glucose, and 10% FBS)/70°C (44).

Two-dimensional electrophoresis: All samples were embedded in Epon resin using standard methods. Ultrathin (80-nm) sections were cut using a diamond knife mounted on a Lecia UC6 ultramicrotome, picked up on 200 mesh copper grids, and stained with uranyl acetate (20 min) and lead citrate (10 min). Sections were examined in a Tecnai 12 BioTwin transmission electron microscope (FEI Electron Optics) using an acceleration voltage of 120 kV and an objective aperture of 20 μm. Digital images at various magnifications were acquired with a MegaView 3 camera (Soft Imaging Systems).

Experimental infections. Experimental infections were carried out under license from the Department of Health and Children as part of a multidisciplinary study of the immunopathogenesis of EAE. Sixteen seronegative pregnant ewes (90 days of gestation) sourced from flocks with no known history of EAE were challenged by subcutaneous injection with 2 × 10⁶ IFU/ml of C. abortus cultured in yolk sac; eight control pregnant ewes were inoculated with uninfected yolk sac. Blood samples were collected weekly from each ewe from day 0 until time of parturition. Blood samples were allowed to clot; sera were collected and stored at −20°C.

One-dimensional SDS-PAGE and immunoblotting. One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the discontinuous buffer system as described by Laemmli (17). Purified RB and EB were washed twice with 50 mM Tris-HCl, pH 6.8, and solubilized in 50 mM Tris-HCl containing 1% SDS. Before electrophoresis, samples were mixed with an equal volume of 2× sample loading buffer and boiled for 5 to 10 min before loading. Total proteins were visualized after staining with silver (PlusOne Silver staining kit; GE Healthcare, United Kingdom). Alternatively, immunoblotting was performed with eewas as follows: separated proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore Ireland B.V.) by semidy transfer (Pharmacia Biotech, GE Healthcare, United King-
dom) for 1 h 30 min at 45 mA. Free binding sites on membranes were blocked with 5% low-fat milk powder in PBS-0.1% Tween 20 (PBS-T) for 1 h at room temperature or at 4°C overnight. After blocking, the membranes were incubated with horseradish peroxidase (HRP) conjugate (1-2,000 in PBS) or fetal serum (1:500 in PBS), washed thrice with PBS-T, and incubated for 2 h with donkey anti-sheep IgG-horseradish peroxidase (HRP) conjugate (1-2,500) (Sigma-Aldrich Ireland Ltd.). Bound conjugates were detected with SuperSignal West Dura extended-duration substrate (West Pico reagent; Pierce, Thermo Fisher Scientific Inc., United Kingdom) for 5 min. Images were visualized with the BioSpectrum AC imaging system (Ultra-Violet Products Ltd., United Kingdom).

Two-dimensional electrophoresis. Sample preparation for 2-D gel electrophoresis was performed as previously described (29, 30). Purified EB (75 to 100 μg) was washed twice with Tris-EDTA (TE; 10 mM Tris-Cl, 1 mM EDTA, pH 8.0) buffer and solubilized in lysis buffer (7 M urea, 2 M thiourea [GE Healthcare, United Kingdom], and 1% ASB-14 [amidosulfobetaine-14 [-3-[(N,N-di- methyl-(3-methyl-2-oxoimidazolidine-4-ylmethyl)ammonio)]propanesulfonate]; Sigma-Aldrich, Ireland Ltd.]); vs-Dithiothreitol (DTT) was added to 30 mM prior to loading on to the first dimension (immobilized pH gradient [IPG]), along with the appropriate pH-range IPG buffer to 0.5%. Samples were allowed to swell overnight at room temperature into the immobilized pH gradient strip (7-cm Immobiline DryStrips were used for preparative gels and 18-cm Immobiline DryStrips were used for analysis gels; GE Healthcare, United Kingdom). The isoelectric focusing (IEF) parameters for the 7-cm Immobiline DryStrips were set as recommended (11). The IEF parameters for the 18-cm Immobiline DryStrips were as follows (29): (i) 1 min at a 500-V gradient, (ii) 1.5 h at a 4,000-V gradient, and (iii) 8,000 V for 40,000 Vh, with a 50-μA-per-strip maximum setting at 20°C. After IEF, strips were equilibrated with equilibration buffer (6 M urea, 75 mM Tris-HCl (pH 8.8), 29.3% glycerol, 2% SDS, and bromphenol blue buffer [1% bromphenol blue and 50 mM Tris base]) containing 1% DTT for 10 min and then with equilibration buffer containing 2.5% iodoacetamide for another 10 min. The second dimension was performed using a 12% SDS-PAGE discontinuous buffer system as described by Laemmli (17). Total proteins were visualized after staining with Sypro Ruby protein gel stain (Sigma-Aldrich Inc., Ireland) under UV light in the BioSpectrum AC imaging system (Ultra-Violet Products Ltd., United Kingdom). Alternatively, proteins were transferred to PVDF membranes for 2-D immunoblotting (as described previously for 1-D immunoblotting).

Identification of antigenic proteins. Protein spots identified by Sypro Ruby staining which aligned with reactive antigens of the same molecular mass and isoelectric point were excised with a One Touch 2 gel dot spot picker (3 mm; Web Scientific, United Kingdom). In-gel trypsin digestion was performed as previously described (29). Briefly, gel spots were dehydrated in acetonitrile for 30 min. Acetonitrile was then removed, and the spots were dried in a concentrator (Eppendorf, United Kingdom). Spots were then rehydrated in 100 mM NH₄HCO₃ for 30 min, again dehydrated with acetonitrile for 30 min, and dried in a concentrator. Gel spots were rehydrated with trypsin solution (50 mM NH₄HCO₃, 5 mM CaCl₂, 12.5 ng/μl trypsin) on ice for 45 min. Trypsin solution was then replaced with a solution containing 50 mM NH₄HCO₃, and 5 mM CaCl₂ and incubated at 37°C overnight. After washing with a small volume of 20 mM NH₄HCO₃, peptides were extracted three times with 5% formic acid and 50% acetonitrile for 20 min. A final extraction comprised 100% acetonitrile for 20 min. All extractions were pooled and dried in a concentrator (Eppendorf, United Kingdom). Extractions were stored at −20°C until analysis by mass spectrometry.

Analysis of tryptic peptide sequence tags by nLC-MS-MS. Samples were analyzed by nano-liquid chromatography–tandem mass spectrometry (nLC-MS-MS) using data-dependent acquisition mode on an LTQ FT ultra mass spectrometer (7 Telsa; Thermo Fisher Scientific, San Jose, CA). After dissolution in 10 μl 0.1% formic acid–1% (vol/vol) acetonitrile, samples were injected onto a trapping column (3 cm, 100 μM, C₁₈, Micro-Tech) previously equilibrated in 100% solution A (0.1% formic acid, 1% acetonitrile in water) at a flow rate of 2 μl/minute. Following a 10-min washing, the trapping column was eluted through a pre-equilibrated analytical column (15 cm, 75 μM C₁₈, Micro-Tech) at a flow rate of 0.300 minutes/minute using a compound linear gradient (3 min at 95% solution A, 8 min at 85% solution A and 15% solution B [0.1% formic acid in acetonitrile], 18 min at 65% solution A and 35% solution B, 30 min at 25% solution A and 75% solution B, and 50 min at 90% solution A and 10% solution B). Column eluent was directed to an uncoated pulled silica nanospray tip (PicoTip FS360-20-10-N-S-C12; New Objective, MA) at 2.4 kV for ionization without nebulizer gas. The mass spectrometer was operated in the data-dependent mode with a precursor survey scan (at m/z 350 to 2000) at 100,000 resolution (at m/z 400), and data-dependent MS-MS was performed in the ion trap for the top 6 precursor ions while employing optimized dynamic exclusion settings in recruiting those ions. Individual sequencing experiments were matched to a protein sequence database of Chlamydia abortus S26/3 using Mascot (Matrix Science, United Kingdom) and the Inspect (interpretation of spectra with post-translational modifications) tool (9, 42). The search was run using trypsin as the default enzyme and variable modification of methionine oxidation.
RESULTS

EB. Since *C. abortus* has a biphasic development cycle comprising RB and EB, purified EB preparations were examined by transmission electron microscopy to ensure sample purity (Fig. 1B). The phenotypic differences between RB and EB observed by transmission electron microscopy were supported by differential protein expression as detected by 1-D SDS-PAGE (Fig. 2A). The importance of working with the infectious EB is further evidenced by the large number of antigens which are reactive with serum from an experimentally infected ewe, compared to a single antigen of 26 kDa that is reactive in purified RB, despite the large numbers of proteins present (Fig. 2B).

Analysis of sera from experimentally infected ewes and fetuses by immunoblotting. 1-D gel immunoblot analysis of EB with ovine sera confirmed that all infected ewes had seroconverted by 14 dpi (Fig. 3). No significant reactivity with chlamydial antigens was detected using sera from control ewes or sera collected at day 0 from experimentally infected ewes. Two antigens, of approximately 90 and 40 kDa, were reactive with sera from 100% (16/16) of infected ewes. Immunoblotting was also performed using sera from experimentally infected ewes collected at weekly intervals postinfection (Fig. 4). It was possible to observe an increase in the number and intensity of antigens detected over time. Antibodies to antigens of approximately 26, 28, and 59 kDa were identified in the sera of 12/16 (75%), 14/16 (87.5%), and 12/16 (75%) ewes, respectively, by 14 dpi.

Sera from the fetuses of experimentally infected ewes were also specific for antigens of EB of *C. abortus*. No chlamydial antigens were reactive with sera from control fetuses or with sera taken from fetuses at 30 dpi. Antigens were first detected with sera from fetuses taken at 35 dpi (Fig. 5). At 43 dpi, 71% (5/7) of fetal sera reacted with a 26-kDa antigen, and 43% (3/7) reacted with a 59-kDa antigen.

Two-dimensional electrophoresis of EB. The proteome of EB of *Chlamydia abortus* was separated by 2-D gel electrophoresis over a pH range of 3 to 10 (Fig. 6A). Since the majority of detectable antigens of EB had an isoelectric point between 4 and 7, proteins were also separated over a pH range of 4 to 7 to provide increased resolution (Fig. 6B). More than 100 spots were detected in these gels, with molecular masses between 220 kDa and <20 kDa. A highly abundant protein

FIG. 1. Transmission electron microscopy of *Chlamydia abortus*. (A) RB (arrows), 1 μm in diameter, within a McCoy cell at 24 h postinfection. (B) Purified EB (arrows) harvested from McCoy cells at 72 h postinfection, 300 to 500 nm in diameter.

FIG. 2. One-dimensional SDS-PAGE and immunoblotting of 1 μg of RB and EB from *Chlamydia abortus*. Silver stain of total protein (A) and immunoblot (B) (serum sample from ewe 8 collected 34 dpi, 1:2,000). Lane 1, negative control (mock purification of RB from noninfected McCoy cells); lane 2, negative control (mock purification of EB from noninfected McCoy cells); lane 3, purified RB; lane 4, purified EB. Molecular mass (kDa) standards are indicated on the left. Asterisks indicate protein bands unique to RB, while arrows indicate protein bands unique for EB.

FIG. 3. One-dimensional immunoblot of elementary bodies of *C. abortus*. Membranes were probed using sera (dilution of 1:2,000) from the 16 infected ewes collected at day 14 postinfection. (A) Ewes 1 to 8; (B) ewes 9 to 16. Molecular mass (kDa) standards are indicated on the left. Arrows indicate reactive antigens at 40 kDa and 90 kDa.
Antigens of Chlamydia abortus

with multiple isoforms was detected with a pl between 5 and 8 and an apparent molecular mass of ~40 kDa (Fig. 6A, circled). Based on results obtained from 1-D immunoblotting, serum from an experimentally infected ewe (ewe 8, 34 dpi) was selected for immunoblot analysis of EB separated by 2-D gel electrophoresis (Fig. 6C and D). More than 30 antigens were detected by 2-D immunoblotting, significantly more than were detected by 1-D immunoblotting. Highly reactive antigens of 26 to 32 kDa with a pl between 4 and 5 were detected (Fig. 6C, square box). However, examination of the SYPRO Ruby-stained gels (Fig. 6A) revealed that barely detectable quantities of proteins were present in the same region. Other highly reactive antigens detected included a protein with an apparent molecular mass of approximately 40 kDa and a pl between 6 and 7.5 (Fig. 6C, circled), which appears to correspond to the highly abundant protein detected in the SYPRO Ruby-stained gels (Fig. 6A, circled). Several other reactive antigens with apparent molecular masses of 40 to 120 kDa and pl values between 4.5 and 5.5 were also detected.

Since the reactivity of individual fetal sera was low, a pool of sera from several fetuses was used for 2-D gel immunoblot analysis of EB separated over a pH range of 4 to 7 (Fig. 7). The number of antigens detected increased to more than 20, in contrast to the few detected by 1-D immunoblot. Results showed an antigenic profile similar to that observed using sera from experimentally infected pregnant ewes (Fig. 6D).

Identification of antigenic proteins by mass spectrometry.

Two-dimensional immunoblots were aligned with corresponding protein-stained gels, and proteins were excised for identification by mass spectrometry (Fig. 6B and D). In order to identify those antigens of 26 to 32 kDa with a pl between 4 and 5, which did not have detectable protein in 2-D gels containing 75 μg of EB proteins separated over pH 3 to 10 (Fig. 6A), 500 μg of protein was separated over pH 4 to 7 (Fig. 6B). Proteins which were excised and identified by mass spectrometry are numbered and listed in Table 1 (see also Table S1 in the supplemental material). Proteins with functions related to cell shape, transport, protein folding, biosynthesis, and other metabolic processes were identified. Spots 1 and 2 were identified as being the polymorphic outer membrane protein Pmp18D (CAB776), while spots 3 and 4 were identified as major outer membrane protein precursors (CAB048). Several spots had more than one significant hit. Spots 5 to 9 all gave significant hits for the putative macrophage infectivity potentiator lipoprotein (CAB080). Spots 5 and 6 also had hits for a cysteine-rich outer membrane protein and a putative UDP-N-acetylglucosamine acyltransferase, respectively. Both spots 8 and 9 had hits for putative inorganic pyrophosphatase. Spot 8 also had a significant hit as a putative lipoprotein. Spot 10 had significant hits for putative elongation factor and an ABC transporter, substrate binding lipoprotein.

**DISCUSSION**

Chlamydia abortus is a leading cause of ovine abortion in Ireland and the United Kingdom. In order to emulate as closely as possible the naturally occurring disease process, pregnant ewes were inoculated with $2 \times 10^6$ inclusion forming units of C. abortus. In this way, sera from a clinically relevant experimental infection were used to identify immunologically relevant proteins of C. abortus that are expressed during ovine infection.

C. abortus has a biphasic life cycle comprising elementary and reticulate bodies. Elementary bodies are the extracellular infectious form compared to the metabolically active intracellular reticulate bodies. The phenotypic differences between purified EB and purified RB were apparent by transmission electron microscopy and confirmed by differential protein expression by 1-D SDS-PAGE. As expected, several proteins of EB were reactive with sera from infected ewes compared to RB. Results highlight the specific host interactions with infectious EB during disease processes, compared with the paucity of reactivity of sera with intracellular RB.

All experimentally infected pregnant ewes had seroconverted by day 14, and all reacted specifically with antigens from purified EB of 40 and 90 kDa, compared to negative controls (Fig. 3). The 40-kDa antigen was also evident in 2-D immunoblots in several isoforms and, when excised from protein gels, was subsequently identified as the major outer membrane protein (MOMP). This provides an excellent positive control.
for our experimental design, as MOMP is a well-characterized outer membrane protein from *C. abortus* and is also currently used in diagnostic assays (52). While a 90-kDa antigen is apparent on 2-D immunoblots of EB separated over a pH of 3 to 10, it was not detected on immunoblots separated over a pH range of 4 to 7. Previous studies have identified 90-kDa antigens as the polymorphic outer membrane proteins (12, 47). However, while not identified at 90 kDa, the polymorphic outer membrane protein CAB776, which has a predicted molecular mass of 163 kDa, was reactive with sera from experimentally infected ewes and identified at an apparent molecular mass of 50 kDa; this is likely a POMP breakdown product and is supported by the mass spectrometry results which showed that the identified peptides were derived from the C terminus of the POMP (see Table S1 in the supplemental material).

FIG. 6. Two-dimensional gel electrophoresis and immunoblotting of elementary bodies of *C. abortus*. (A) A total of 75 μg of protein SYPRO Ruby stained, 3 to 10 pH range, 18-cm Immobiline DryStrip. (B) A total of 500 μg of protein SYPRO Ruby stained, 4 to 7 pH range, 18-cm Immobiline DryStrip. (C) A total of 75 μg of protein immunoblotted with serum from a selected ewe (ewe 8, 34 dpi), dilution 1:1,000, 3 to 10 pH range, 18-cm Immobiline DryStrip. Square box indicates the 26- to 32-kDa reactive antigens; the circle indicates the ~40-kDa reactive antigens. (D) A total of 100 μg of protein immunoblotted with serum from a selected ewe (ewe 8, 34 dpi), dilution 1:1,000, 4 to 7 pH range, 18-cm Immobiline DryStrip. Circles and numbers shown in panels B and D indicate the excised protein spots identified by mass spectrometry (Table 1). Molecular mass standards (kDa) are indicated on the left.

FIG. 7. Two-dimensional immunoblotting of elementary bodies of *C. abortus*. A total of 20 μg of elementary body proteins (4 to 7 pH range, 7-cm Immobiline DryStrip) were probed with sera from a pool of fetal sera (dilution 1:500). Molecular mass (kDa) standards are indicated on the left.
nogenic protein detected at day 43 postinfection was at an apparent molecular mass of 26 kDa. Similarly, sera from 75% and 93.8% of infected ewes were reactive with a 26- and 28-kDa antigen, respectively. No fetal antibodies specific for EB were detected at 30 dpi. The failure of the fetuses to respond serologically to EB (22, 24, 31, 35). Further, the recombinant MIP of C. trachomatis induced release of the proinflammatory cytokines IL-6, and IL-8 in human monocytes/macrophages through Toll-like receptor 2 (TLR2)/TLR1/TLR6 and CD14, suggesting a significant role for MIP in the pathogenesis of C. trachomatis-induced inflammatory responses (3). MIP has also been identified in other intracellular bacteria, including Legionella pneumophila, in which it is reported to facilitate dissemination within host tissues, as demonstrated by transmigration of lung epithelial cells due to a serine protease activity and specific interaction with collagen (50).

Additional antigens of EB which are reactive with ovine sera and identified by mass spectrometry include proteins with functions in cell shape, metabolism, lipid synthesis, and transport. The 60-kDa cysteine-rich outer membrane protein (OmcB) maintains the structure of the outer membrane (7, 8) while a putative UDP-N-acetylglucosamine acyltransferase facilitates synthesis of lipid A. A putative lipoprotein was also identified and had 70%, 63%, and 44% similarity with hypothetical proteins of Chlamydo phosphate fels, C. caviae, and C. pneumoniae, respectively, but low similarity (26% and 22%) with proteins from Chlamydia muridarum and C. trachomatis (1, 16, 32, 33, 39, 41). A putative inorganic pyrophosphatase, an enzyme that catalyzes the formation of orthophosphate from pyrophosphate, is highly conserved in both Chlamydia and Chlamydo phila species, with similarities of between 82% and 93% (45). A putative elongation factor Ts (EF-Ts) is essential for cell viability in C. muridarum (54). An ABC transporter was identified and was 66% similar to C. trachomatis TroA, a protein of the chlamydial envelope which is reactive with sera from human patients suffering from infection (2). ABC transporter proteins may be suitable targets for the development of antibacterial vaccines, postinfection therapies, or the development of novel antimicrobials which exploit ABC transport systems (10).

Experimental infection of pregnant ewes with C. abortus emulates clinically relevant disease processes. Alternative routes of infection, including intranasal and oral challenges, have recently been validated and may facilitate the identification of additional antigens expressed during infection (13, 25).
This might explain why the infected fetus reacts with different antigens compared to the ewe, since it was infected in utero. The identification of the macrophage infectivity potentiator protein as being highly reactive with sera from both infected ewes and fetuses relative to other proteins suggests that it plays a significant role during pathogenesis and is worthy of further investigation as a diagnostic antigen.

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