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(71) Applicants (for all designated States except US): **UNIVERSITEIT GENT** [BE/BE]; St. Pietersnieuwstraat 25, B-9000 Gent (BE). **DE BELGISCHE STAAT VERTEGENWOORDIGD DOOR FEDERALE OVERHEIDSDIENST VOLKSGEZONDHEID, VEILIGHEID VAN DE VOEDSELKETEN EN LEEFMILIEU** [BE/BE]; Eurostation II, Victor Hortaplein 40, bus 10, B-1060 Brussel (BE).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **VANROMPAY, Daisy** [BE/BE]; Houte 101B, B-9860 Oosterzele (BE).

(74) Agent: **LAENEN, Bart**; LC Patents, Crutzenstraat 26, B-3500 Hasselt (BE).

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[Continued on next page]

(54) Title: VACCINES FOR *CHLAMYDIA*

Total antibody titer against a recombinant protein of *C. psittaci* in pharyngeal mucosal swabs

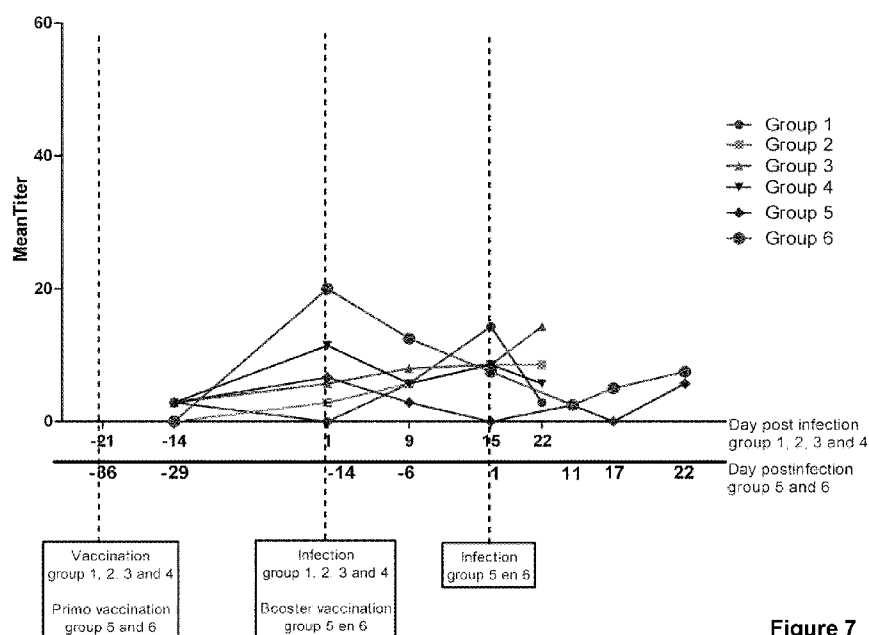


Figure 7

(57) Abstract: The present invention relates to means and methods to protect against disease caused by bacteria belonging to the genus *Chlamydia*. In particular, the present invention relates to isolated B- and T-cell epitopes derived from the major outer membrane protein of *Chlamydia psittaci* which can be used against an infection with a species of the genus *Chlamydia*. More in particular, the invention provides a vaccine which can be used against chlamydiosis caused by *Chlamydia psittaci* in birds and man. In addition, the invention relates to a diagnostic method to diagnose the latter infections.

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Vaccines for *Chlamydia*

Field of the invention

The present invention relates to means and methods to protect against disease caused by bacteria belonging to the genus *Chlamydia*. In particular, the present invention relates to isolated B- and T-cell epitopes derived from the major outer membrane protein of *Chlamydia psittaci* which can be used against an infection with a species of the genus *Chlamydia*. More in particular, the invention provides a vaccine which can be used against chlamydiosis caused by *Chlamydia psittaci* in birds and man. In addition, the invention relates to a diagnostic method to diagnose the latter infections.

Background art

The genus *Chlamydia* comprises the species *Chlamydia abortus*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Chlamydia pecorum*, *Chlamydia felis*, and *Chlamydia caviae*. Diseases caused by bacteria belonging to the genus *Chlamydia* are the following. *Chlamydia pneumoniae* causes acute or chronic bronchitis and pneumonia in humans and in some cases otitis media, obstructive pulmonary disease and pulmonary exacerbation of cystic fibrosis. It has also been associated with Alzheimer's disease, atherosclerosis, asthma, erythema nodosum, reactive airway disease, Reiter's syndrome and sarcoidosis in humans. Moreover, *Chlamydia pneumoniae* causes respiratory disease in koalas. *Chlamydia pecorum* strains characterized so far have been limited to mammals, but not to a specific host family. They are serologically and pathologically diverse. The organism has been isolated from ruminants, a marsupial and swine. In koalas, *C. pecorum* causes reproductive disease, infertility and urinary tract disease. It has been a major threat to the Australian koala population. In other animals *C. pecorum* causes abortion, conjunctivitis, encephalomyelitis, enteritis, pneumonia and polyarthritis. *Chlamydia felis* is endemic among house cats worldwide. It causes primarily conjunctivitis and rhinitis. *Chlamydia caviae* comprises five known isolates, all isolated from guinea pigs. The natural infection site is the mucosal epithelium of the conjunctiva where a non-invasive infection is established. However, it is possible to infect the genital tract of guinea pigs eliciting a disease that is very similar to human genital infection. *Chlamydia abortus* strains are endemic among ruminants and efficiently colonize the placenta. *Chlamydia abortus* is the most common infectious cause of abortion in sheep, where the disease is known as ovine enzootic abortion (OEA) or enzootic abortion of ewes (EAE) in countries of Northern, Central and Western Europe. Enzootic abortion in goats is similar in

severity to that occurring in sheep, although the spread and economical impact across Europe is less clear because of the lack of epidemiological data. The disease can also affect cattle, swine and horses but this is thought to occur to a much lesser extent. Sporadic zoonotic abortion due to *C. abortus* has been confirmed by analysis of isolates from women who work with sheep and goats. *Chlamydia psittaci* produces avian respiratory infections and is a serious threat to industrial poultry production. Additionally, it causes epizootic outbreaks in mammals and respiratory psittacosis or parrot fever in humans.

Protective immunity to *Chlamydiaceae* (including the genus *Chlamydia*) that is observed in animals previously exposed to the pathogen is believed to be effected primarily through the action of CD4⁺ T helper type 1 (Th1) lymphocytes, CD8⁺ T lymphocytes, mononuclear phagocytes, and cytokines secreted by these cells (Cotter et al., 1995; Su and Caldwell, 1995; Beatty et al., 1997; Cotter et al., 1997; Johansson et al., 1997; Su et al., 1997; Williams et al., 1997; reviewed by Kelly et al., 2003).

Initial attempts to develop an effective vaccine for controlling both animal and human chlamydial infections began with the use of inactivated or live whole organism preparations in the 1950s, e.g. for *C. abortus* in sheep. In general, such preparations offered a reasonable level of protection, although they have been more successful in protecting animal infections than human infections (Vanrompay et al., 2005).

During the 1960s unsuccessful attempts were made to develop inactivated and live vaccines against *C. trachomatis* using both human and non-human primates. These vaccines reduced disease in some individuals but they enhanced disease in others resulting from stimulation of enhanced delayed-type hypersensitivity (DTH) response. Therefore, the use of whole organisms for developing human chlamydial vaccines was essentially abandoned.

In the early 1980s an attenuated strain of *C. abortus* was developed as a live vaccine and is one of the 5 commercially available vaccines in Europe and the USA, the other four being inactivated whole organism based vaccines (Longbottom and Livingstone, 2006). These commercial live-attenuated and inactivated vaccines offer good protection against OEA and significantly reduce the shedding of infective organisms, a factor important in limiting the spread of infection to other animals. However, concerns remain over the safety of using live-attenuated vaccines. There may also be a risk of the attenuated strain reverting to virulence, thus having the potential to cause disease and abortion in the vaccinated animal. Furthermore, the vaccine cannot be administered during pregnancy or to animals being treated with antibiotics limiting its use. In contrast, the inactivated vaccines can be administered to

pregnant ewes, although care must be taken in handling and administering these vaccines as they are adjuvanted with mineral oils, which have the potential to cause tissue necrosis if accidentally self-injected. The only other animal chlamydial vaccines, which are commercially available are for *C. felis* infection in cats (Longbottom and Livingstone, 2006).

5 Although vaccination is successful in reducing acute disease, it does not, however, prevent shedding of the organism and therefore chlamydial spreading in the population nor does it prevent re-infection. Following the identification of the major outer membrane protein (MOMP) as a structurally and immunologically dominant protein vaccine research largely focused on this protein. A certain level of protection has been achieved with COMC
10 (chlamydial outer membrane complex) preparations, in which MOMP constitutes 90% or more of the protein content, using the guinea pig and mouse models for *C. trachomatis* genital tract infection, and in a mouse toxicity test for *C. felis* infection (Sandbulte et al., 1996; Pal et al., 1997). Studies on MOMP of *C. psittaci* were performed e.g. by Tan et al., 1990, Sandbulte et al., 1996 and Verminnen et al., 2005.

15 DNA vaccination, mimicking a live vaccine, creates protective CD4⁺ as well as CD8⁺ responses but antibody responses are low (Verminnen et al., 2010). Moreover DNA vaccines are still too expensive (especially for poultry) and the public is not (yet) ready to consume products from DNA vaccinated animals (in the case of *C. psittaci* eggs/meat).

20 Until now, completely safe and effective *Chlamydia* vaccines are still not available.

The present invention comprises an innovative vaccine that creates both optimal humoral and cellular immune responses by combining B cell and CD4 Th2 cell epitopes (humoral) and
25 CD4 Th1 and CD8⁺ cytotoxic T cell epitopes (cellular) in a vaccine. In contrast, former and current studies on *Chlamydia* vaccine development are focusing on cellular responses only (CD8 and CD4 Th1), as they are believed to be crucial to protection against this obligate intracellular pathogen (Su and Caldwell, 1995; Morrison et al., 2000). Moreover, due to the Th1/Th2 paradigm (both responsible for different types of protective responses) vaccine
30 development against infectious agents focuses on creating either a Th1 or Th2 response (Th1 or Th2 polarized vaccines). Thus, the construction of a non-polarized vaccine, deliberately inserting Th1 as well as Th2 epitopes is highly unusual and new as Th1 and Th2 cells act differently and even opposing.

The present invention relates to selected protective B-and T- (Th1 and Th2) cell epitopes of an immunodominant protein (the major outer membrane protein or MOMP). Such protein-based vaccines can be used against an infection with a species of the genus *Chlamydia*. These peptide sequences do not cause immunopathological reactions nor immunosuppressive T cell responses. Reversion to virulence is not applicable, thus enhancing the safety for both animals and humans. The epitopes can be used in any suitable vector, preferably a vector which: a) can be used in the presence of *species*-specific maternal antibodies and b) allows easy and cost effective vaccine mass production. Moreover, and surprisingly, the present invention demonstrates that both the cellular- and humoral immune response are required to protect against an infection with a species of the genus *Chlamydia*.

Figure legends

Figure 1: structure of the MOMP located in the bacterial membrane

Figure 2: detailed structure of the MOMP. Solid line, membrane-embedded peptide chain of MOMP; solid line, 5 conserved sequences; pointed squares, residues comprising the variable sequences (VSs) I, II, III and IV; open squares, conserved cysteines. The presence of lipopolysaccharide (LPS) structures (solid blocks) is indicated above the outer membrane (OM). Claimed B cell epitopes are in VSI (immunodominant), VS2 (serovar) and VS4 (immunodominant). Claimed T cell epitopes are in CS1, CS4 and VS4.

Figure 3: (A.) B cell epitope mapping results for the *C. psittaci* serovar D strain 92/1293 MOMP using the serovar D-specific MAb NJ1 (1/100); (B.) B cell epitope mapping results for the *C. psittaci* serovar D strain 92/1293 MOMP using serum of SPF turkeys immunized with recombinant MOMP. VSI: peptides 1 to 20, VSII: peptides 21 to 32, VSIII: peptides 33-40, VSIV: peptides 41 to 67.

Figure 4: Alignment of *Chlamydia* and *C. psittaci* ompA nucleotide sequences and MOMP amino acid sequences.

Lines: 1) *Chlamydia trachomatis* (Ctra), 2) *Chlamydia muridarum* (Cmur), 3) *Chlamydia pneumoniae* (Cpn), 4) *Chlamydia felis* (Cfel), 5) *Chlamydia caviae* (Ccav), 6) *Chlamydia abortus* (Cabo). Lines 7 till 18: *Chlamydia psittaci* strains 84/2334, 6BC, CP3, GD, NJ1, 92/1293, Cal10, VS225, WSRTE30, M56 and WC. The *ompA* and MOMP sequence of reference strain 92/1293 used for vaccine design is underlined.

Figure 5: Gross pathology.

Figure 6: Mean serum antibody titers against a recombinant protein (MOMP) of *C. psittaci*.

The ELISA plates were coated with recombinant MOMP of *C. psittaci*.

Figure 7: Mean mucosal antibody titers against a recombinant protein (MOMP) of *C. psittaci*.

The ELISA plates were coated with recombinant MOMP of *C. psittaci*.

Description of the invention

5 The present invention relates to the use of isolated peptides comprising B- and T-cell epitopes for producing a vaccine against species of the genus *Chlamydia*, and more in particular to induce an immunoprotective response against an infection with a species of the genus *Chlamydia*.

10 The present invention relates to a composition comprising at least one isolated peptide comprising a B-cell epitope located in a variable amino acid region of the major outer membrane protein (MOMP) of *Chlamydia psittaci* and at least one isolated peptide comprising a T-cell epitope located in a conserved or variable amino acid region of the MOMP of *Chlamydia psittaci*. In particular, the invention relates to a composition comprising one or more peptides, each of said peptides comprising one or more epitopes selected from
15 the list consisting of a B-cell epitope, a CD4⁺ Th2 cell epitope, a CD4⁺ Th1 cell epitope and a CTL epitope; wherein said composition comprises at least a B-cell epitope, a CD4⁺ Th2 cell epitope, a CD4⁺ Th1 cell epitope and a CTL epitope, and wherein the epitopes are located in the major outer membrane protein (MOMP) of *Chlamydia psittaci* and wherein the composition does not comprise the full-length *Chlamydia* MOMP protein.

20 The composition is specifically designed to induce an immunoprotective response against an infection with a species of the genus *Chlamydia*. In particular, the combination of the B- and T-cell epitope containing peptides is not equal to the complete or full-length MOMP protein, in particular the *Chlamydia psittaci* MOMP protein. More particular, the B-cell epitope is located in the variable region I, II or IV and the T-cell epitope (including CD8⁺, CD4⁺ Th1, and CD4⁺ Th2 cell epitopes) is located in the conserved region I or IV, or in variable region
25 IV. The MOMP protein of *Chlamydia psittaci* is described by Vanrompay et al., 1998 (p5496 figure 1, variable domains are indicated).

With the term “B cell-epitope” is meant a part of an antigen that induces antibody production upon recognition by the host’s immune system.

30 A “T-cell epitope” is a part of an antigen that induces a CD4⁺Th1 (T helper, HTL), CD4⁺ Th2 (T helper, HTL) or CD8⁺ cell (cytotoxic, CTL) response upon recognition by the host’s immune system. T helper (Th) cells are considered major players in the response against infectious organisms. To convey their full function, Th cells secrete a variety of cytokines, which define their distinct actions in immunity. Th cells can be subdivided into three different

types based on their cytokine signature, Th1, Th2 and Th17 cells. "Th1 cells" secrete IFN- γ (pro-inflammatory cytokine), which is the main macrophage-activating cytokine and TNF- β , which also activates macrophages, inhibits B cells and is directly cytotoxic for some cells. Th1 cells allow the production of IgG_{2a} antibodies in mice and of IgM, IgA, IgG₁, IgG₂ and IgG₃ antibodies in humans. "Th2 cells" secrete IL-4, IL-5, IL-6, IL-9 and IL-13 all of which activate B cells, and IL10 (important anti-inflammatory cytokine), which inhibits macrophage activation. Th2 cells induce IgG₁ and IgE antibodies in mice and IgM, IgG₄ and IgE in humans. Th17 cells secrete the pro-inflammatory cytokine IL-17 (Murphy et al., 2008; Annunziato and Romagnani, 2009).

A peptide comprising a CTL epitope (CD8⁺) usually consists of 13 or less amino acid residues in length, 12 or less amino acids in length, or 11 or less amino acids in length, preferably from 8 to 13 amino acids in length, most preferably from 8 to 11 amino acids in length (i.e. 8, 9, 10, or 11). A peptide comprising a HTL epitope (CD4⁺) consists of 50 or less amino acid residues in length, and usually from 6 to 30 residues, more usually from 12 to 25, and preferably consists of 12 to 20 (i.e. 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids in length. Peptides comprising B cell epitopes do not have a defined length and can vary from 5 to 30 amino acids in length, preferably from 5 to 15 amino acids in length, i.e. 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids.

In a specific embodiment, a peptide comprising a T cell epitope has a length of 6 to 50 amino acids and a peptide comprising a B cell epitope has a length of 5 to 30 amino acids.

Hence, it is to be understood that peptides with a defined length and comprising the epitopes specified herein are part of the present invention and can be used in the compositions and methods as described herein. In a specific embodiment, the peptides are immunogenic peptides, i.e. peptides capable of eliciting an immune response in an organism, including a cellular and/or humoral response. More particular, the present invention comprises an innovative vaccine that creates both humoral and cellular immune responses by combining B cell and CD4 Th2 cell epitopes (humoral) and CD4 Th1 and CD8⁺ cytotoxic T cell epitopes (cellular) in a composition.

The term "isolated" is used to indicate that a cell, peptide or nucleic acid is separated from its native environment. Isolated peptides and nucleic acids may be substantially pure, i.e. essentially free of other substances with which they may bound in nature.

With the term 'induction of an immunoprotective response' is meant a (humoral and/or cellular) immune response that reduces or eliminates one or more of the symptoms of disease,

i.e. clinical signs, lesions, bacterial excretion and bacterial replication in tissues in the infected subject compared to a healthy control. Preferably said reduction in symptoms is statistically significant when compared to a control.

- 5 Infections with a species of the genus *Chlamydia* are infections with one or more of the species selected from the group consisting of: *Chlamydia abortus*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Chlamydia pecorum*, *Chlamydia felis*, and *Chlamydia caviae*.

10 In a particular embodiment, the present invention provides peptides suitable for vaccine design. Suitable peptides comprising T-cell epitopes correspond to SEQ ID NO 1, 7, 8, 9, 10, 11, 12, 13, 18, 20, 21, 22, 24, 25, 26, 27, 29, 30, 31, 32, 35, 41, 42, 43, 44, 46, 49, 50, 51, 53, 54, 55, 56, 60, 61, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, and 91. As can be derived from the data in Table 1, said peptides are characterized as follows:

- 15 -Peptides comprising a CTL (CD8⁺) epitope correspond to SEQ ID NO 20, 26, 27, 42, 43, 61, 73, 75, and 80.
 -Peptides comprising a CD4⁺ Th1 cell epitope correspond to SEQ ID NO 1, 10-13, 18, 20, 25, 27, 29, 31-32, 35, 42, 46, 51, 53-56, 61, 64-67, 69-73, 75, 79-81, and 87.
 -Peptides comprising a CD4⁺ Th2 cell epitope correspond to SEQ ID NO 7, 8, 43, 76, 77, 85,
 20 and 91.

In addition, peptides comprising a B-cell epitope correspond to SEQ ID NO 32-33, 45-46 and 90-96.

- 25 The composition of the present invention preferably comprises specific combinations of said peptides thereby inducing a humoral as well as a cellular immune response. The composition therefore comprises one or more peptide(s) comprising at least one B cell epitope, at least one CD4 Th2 cell epitope, at least one CD4 Th1 cell epitope and at least one CD8⁺ cytotoxic T cell epitope.

30

More specifically, the present invention relates to the composition as indicated above, wherein said isolated B cell epitope consists of the amino acid sequence GTASATT (SEQ ID NO 92), GTDFNN (SEQ ID NO 93) or NPTLLGKA (SEQ ID NO 94), or variants thereof, which are located in the variable domain (VS) I, II and IV, respectively of the MOMP of

Chlamydia psittaci serovar D strain 921/1293, and, wherein said isolated T-cell epitope consists of the amino acid sequence

selected from the group consisting of:

DGTMWEGAS (SEQ ID NO 101; CD4Th2);

5 ASGDPCDPC (SEQ ID NO 102; CD4Th1);

ATDTKSATLKYHE (SEQ ID NO 103; CD4Th1);

SATLKYHEWQVGL (SEQ ID NO 104; CD4Th1);

SATLKYHEWQVGLAL (SEQ ID NO 105; CD4Th1);

TLKYHEWQVGLAL (SEQ ID NO 106; CD4Th1 and CD8 (CTL));

10 KYHEWQVGLALSY (SEQ ID NO 107; CD4Th1 and CD8 (CTL));

YHEWQVGLALSYR (SEQ ID NO 108; CD4Th2);

HEWQVGLALSYRL (SEQ ID NO 109; CD4Th2);

QVGLALSYRLNM (SEQ ID NO 110; CD4Th2);

RLNMLVPYIGVNW (SEQ ID NO 111; CD4Th1);

15 RLNMLVPYIGVNWS (SEQ ID NO 112; CD4Th1 and CD8 (CTL));

NMLVPYIGVNWSR (SEQ ID NO 113; CD4Th1);

MLVPYIGVNWSRA (SEQ ID NO 114; CD4Th1);

YIGVNWSRAT (SEQ ID NO 115; CD4Th1);

TAVLDLTTW (SEQ ID NO 116; CD4Th2);

20 TTVDGTNTYSDFL (SEQ ID NO 117; CD4Th2); and

TWCDAISIRAGYYGD (SEQ ID NO 20; CD4Th1 and CD8 (CTL));

or variants thereof, which are located in the conserved region I or IV, or in the variable domain IV, of the MOMP of *Chlamydia psittaci* serovar D strain 921/1293. Optionally, flanking amino acids or regions of the specified B- and T- cell epitopes can be part of the

25 peptide sequences to be included in the composition.

In a specific embodiment, the invention relates to the composition and use as indicated above, wherein the peptide comprising a B-cell epitope is selected from the group consisting of:

TGTASATT (SEQ ID NO 95), KGTDFFNNQ (SEQ ID NO 96) and NPTLLGKA (SEQ ID NO 94), or a variant thereof, and wherein the peptide comprising a T-cell epitope comprises

30 or consist of an amino acid sequence selected from the group consisting of: SEQ ID NO 1, 7-13, 18, 20-22, 24-27, 29-32, 35, 41-44, 46, 49, 50, 51, 53-56, 60, 61, 63-82, 84-87, and 91, or a variant thereof. In a particular embodiment, the peptide comprising the B-cell epitope

NPTLLGKA (SEQ ID NO 94) and the CD4 Th2 cell epitopes TAVLDLTTW (SEQ ID NO

116) and TTVDTNTYSDFL (SEQ ID NO 117) is

AQPKLATAVLDLTTWNPTLLGKATTVDGTNTYSDFL (SEQ ID NO 98).

In a preferred embodiment the peptide comprising a T-cell epitope is selected from the group consisting of the amino acids at position:

- 5 30-44, 35-49, 36-50, 37-51, 257-271, 262-276, 263-278, 264-279, 266-280, 267-281, 268-282, 269-283, 279-293, 280-294, 281-295, 282-296, 287-302, and 326-340 of the 356 amino acids MOMP of *Chlamydia psittaci* serovar D strain 921/1293, or variants thereof. The *Chlamydia psittaci* serovar D strain 921/1293 is described by Vanrompay et al., 1998 and in Figure 4.
- 10 The compositions and methods of the present invention also encompass variants of the above specified peptides comprising the epitopes. "Variants" of the B and T-cell epitopes on the corresponding peptide sequences of the different strains or species are also part of the invention, i.e. those peptide sequences at corresponding amino acid positions when aligned to a reference sequence (e.g. Figure 4 for different *Chlamydia* species and for different *Cp.*
- 15 *psittaci* strains with strain 92/1293 being reference sequence). Moreover, a "variant" as used herein, is a peptide that differs from the native antigen only in conservative substitutions and/or modifications, such that the ability of the peptide to induce an immune response is retained. Peptide variants preferably exhibit at least about 70%, preferably at least about 80%, more preferably at least about 90% and most preferably at least about 95% identity to the
- 20 identified peptides. Alternatively, such variants may be identified by modifying one of the above peptide sequences and evaluating the immunogenic properties of the modified peptide using, for example, the representative procedures described herein. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the
- 25 nature of the peptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

30 Variants may also (or alternatively) be peptides as described herein modified by, for example, by the deletion or addition of amino acids that have minimal influence on the immunogenic properties, secondary structure and hydropathic nature of the peptide.

The invention more specifically relates to the composition and use as indicated above, wherein said isolated B-cell epitopes and T-cell (Th2) epitopes are part of a fragment of the 356 amino acid MOMP of *Chlamydia psittaci* serovar D strain 92/1293 which is encoded by

the nucleic acid sequence GCT AGC GAA CCA AGT TTA TTA ATC GAT GGC ACT ATG
TGG GAA GGT GCT TCA GGA GAT CCT TGC GAT CCT TGC ACA GGA ACA GCA
AGT GCT ACT ACT AAA GGA ACT GAT TTC AAT AAT CAA GCT CAG CCT AAA
TTA GCC ACT GCT GTT TTA GAT TTA ACC ACT TGG AAC CCA ACA CTT TTA
5 GGA AAG GCC ACA ACT GTC GAC GGC ACC AAT ACT TAC TCT GAC TTC TTA
GGT ACC (SEQ ID NO 100), and/or, wherein said T cell (Th1) epitopes are part of the
amino acid sequence corresponding to amino-acids 257-301 of the 356 amino acid MOMP of
Chlamydia psittaci serovar D strain 92/1293.

The present invention relates, even more specifically, to the composition or use as indicated
10 above wherein said species of the genus *Chlamydia* is *Chlamydia psittaci*. As such, the
present invention relates to the prevention of morbidity or mortality, or to treatment of
morbidity due to infection with *Chlamydia psittaci* in subjects. Subjects are humans or
animals, but preferably are birds and more preferably poultry, including but not limited to
chickens, ducks, geese and turkeys.

15 A preferred means of administration of the peptides of the present invention is mucosal
delivery, more preferably administration by aerosol or inhalation. Other means of
administration are all other systemic and mucosal administration routes as well as in ovo
administration methods, well known to the skilled person.

20 The composition of the present invention can further comprise a pharmaceutically acceptable
excipient conventional in the art. Non limiting examples of suitable excipients include starch,
glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol
monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water,
ethanol and the like.

25 In the composition, also referred to as polyepitope vaccine, the peptides can be present as a
mixture of individual peptides and/or (part of) the peptides can be linked to each other and/or
are part of vector/carrier construct. In a specific embodiment, the composition comprises a
polyepitope construct. The term polyepitope vaccine as used herein denotes a composition
that does not occur as such in nature. Hence, the "polyepitope vaccine" of the present
30 invention does not encompass a wild-type full-length protein but includes two or more
isolated epitopes of the present invention, not necessarily in the same sequential order or
number (repetitions might be used) as in nature. The polyepitope vaccine of the present
invention preferably comprises 2 or more, 5 or more, 10 or more, 13 or more, 15 or more, 20

or more, or 25 or more epitopes of the present invention. More specific, the polyepitope vaccine comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more epitopes. The epitopes of the polyepitope vaccine can be prepared as synthetic peptides or recombinant peptides. These synthetic peptides or recombinant peptides can be used either individually or directly or indirectly linked to one another. Optionally, two or more of the epitopes (either B-cell and/or T-cell epitopes) can be linked in a construct, referred to herein as a polyepitope construct, and are either contiguous or are separated by a linker or one or more spacer amino acids. "Link" or "join" refers to any method known in the art for functionally connecting epitopes. More particular, the polyepitope vaccine of the present invention is a synthetic or recombinant string of two or more peptides harbouring (part of) the epitopes as described herein. Methods for preparing a polypeptide, which may comprise a polyepitope (polyepitope vaccine/construct), are known in the art and are described in for example the book Molecular Cloning; a laboratory manual by Joseph Sambrook and David William Russell – 2001.

According to a specific embodiment, the composition or polyepitope vaccine of the present invention comprises the following B-cell epitopes: GTASATT (SEQ ID NO 92), GTDFNN (SEQ ID NO 93) and NPTLLGKA (SEQ ID NO 94), or a variant thereof, and the following T-cell epitopes: SEQ ID NO 20 and 101-117, or a variant thereof. In a particular embodiment said B- cell epitopes are comprised in the following peptide sequences: SEQ ID NO 94-96, and said T-cell epitopes in the composition are comprised in the following peptide sequences: SEQ ID NO 7-8, 70, 71, 73, 75-77, 79-81 and 91, or a variant thereof.

In a preferred embodiment, the composition or polyepitope vaccine of the present invention comprises the following peptide sequences:

AA sequence 30-51 (EPSLLIDGTMWEGASGDPCDPC, CD4Th2 epitope in CS1; SEQ ID NO 97), AA sequence 93-100 (TGTASATT, immunodominant B cell epitope in VS1; SEQ ID NO 95), AA sequence 163-170 (KGTDFFNNQ, serovar D B-cell epitope in VS2; SEQ ID NO 96), AA sequence 305-340 (AQPKLATAVLDLTTWNPTLLGKATTVDGTNTYSDFL, CD4Th2 and immunodominant B cell epitope in VS4; SEQ ID NO 98) (the CD4Th2 – B cell cluster), and AA sequence 257-301 (AATDTKSATLKYHEWQVGLALSRLNMLVPYIGVNWSRATFDADT) (CD4Th1 – CD8 cluster in CS4; SEQ ID NO 99), or variants thereof.

In a further preferred embodiment, the composition or polyepitope vaccine of the present invention comprises the following peptide sequences: AA sequence 53-67

(TWCDASIRAGYYGD, CD4Th1 and CD8 epitope; SEQ ID NO 20), AA221-235 (EMLNVTSSPAQFVIH, CD4Th2 epitope; SEQ ID NO 62), and AA163-170 KGTDFFNQ, B-cell epitope; SEQ ID NO 96).

5 The present invention further includes an isolated nucleic acid encoding the epitopes, peptides or polypeptide construct as described herein. Particular nucleic acids encoding the peptides of the invention are the following.

- Corresponding to AA 30-51: CD4Th2 epitope:

5'GAACCAAGTTTATTAATCGATGGCACTATGTGGGAAGGTGCTTCAGGAGATCCTTGCGA
10 TCCTTGC-3' (SEQ ID NO 118)

- Corresponding to AA 93-100: CD4Th1 and immunodominant B-cell epitope:

5'-ACAGGAACAGCAAGTGCTACTACT-3' (SEQ ID NO 119)

- Corresponding to AA 163-170: serovar-specific B-cell epitope:

5'-AAAGGAACTGATTTCATAATCAA-3' (SEQ ID NO 120)

15 - Corresponding to AA 257-301: CD4Th1 + CD8 T cell epitope cluster:

5'GCTGCTACAGATACTAAGTCTGCAACACTCAAATATCATGAATGGCAAGTTGGTCTAGC
ACTCTCTTACAGATTGAACATGCTTGTTCTTACATTGGCGTAAACTGGTCAAGAGCAACT
TTTGATGCTGACACT-3' (SEQ ID NO 122)

- Corresponding to AA 305-340: CD4Th2 epitope + immunodominant B-cell epitope:

20 5'GCTCAGCCTAAATTAGCCACTGCTGTTTTAGATTTAACCCTTGGGAACCCAACACTTTTA
GGAAAGGCCACAACCTGTCGACGGTACCAATACTTACTCTGACTTCTTA-3' (SEQ ID NO
121)

Further embodiments of the present invention are a vector which comprises a nucleic acid
25 encoding at least the polypeptide vaccine or construct as described herein, and which is capable of expressing the respective peptides. A host cell comprising the expression vector and a method of producing and purifying the herein described peptides are also part of the invention. Suitable vectors that can be used in the present invention are known to the skilled
30 in the art and include a plasmid, a bacterial, a viral vector or a yeast vector. Examples of bacterial vectors are Salmonella typhi, BCG (Bacille Calmette Guérin) and Listeria. Examples of viral vectors are poxvirus, Alphaviruses (Semliki Forest Virus, Sindbis Virus, Venezuelan Equine Encephalitis Virus (VEE), Herpes simplex Virus (HSV), Kunjin virus, Vesicular Stomatitis Virus (VSV) replication-deficient strains of Adenovirus (human or simian), polyoma vectors (such as SV40 vectors, bovine polyoma), CMV vectors, papilloma virus

vectors, influenza virus, measles virus, and vectors derived from Epstein Barr virus. A wide variety of other vectors useful for therapeutic administration or immunization, e.g. lentiviral vectors, retroviral vectors, and the like, will be apparent to those skilled in the art. Examples of yeast vectors are a *Hansenula* cell or *Saccharomyces cerevisiae* cell. The composition according to the present invention can further comprise an antigen delivery system, which optimizes the presentation of the antigen. In a specific embodiment, the antigen delivery system is an enzymatically inactive recombinant adenylate cyclase (CyaA) originating from *Bordetella pertussis* (the causative agent of whooping cough) (Ladant et al., 1999; and in EP1576967).

The composition of the present invention can further comprise an adjuvant. Suitable adjuvants are 1) receptor specific (mucosal) adjuvants such as for instance adjuvants binding to pathogen recognition receptors (PRRs) and ganglioside receptor binding toxins, 2) antigen presenting cell targeting (mucosal) adjuvants such as for instance the ones described by Gerdt et al., (2006). Further examples of adjuvants include, but are not limited to, tensoactive compounds (such as Quil A), mineral salts (such as aluminium hydroxide), micro-organism derived adjuvants (such as muramyl dipeptide), oil-in-water and water-in-oil emulsions (such as Freund's incomplete adjuvant), particulate antigen delivery systems (such as liposomes, polymeric atmospheres, nanobeads, ISCOMATRIX) , polysaccharides (such as micro-particulate inulin), nucleic acid based adjuvants (such as CpG motifs), cytokines (such as interleukins and interferons), activators of Toll-like receptors and eurocine L3 en N3 adjuvantia. In a specific embodiment, the adjuvant is an ISCOMTM (ISCOTEC AB, Uppsala, Sweden).

The composition of the present invention can be used as a medicament, and more specific against an infection with a species of the genus *Chlamydia*, preferably wherein said species of the genus *Chlamydia* is *Chlamydia psittaci*. In a further embodiment, the composition is a vaccine. With the term 'vaccine' is meant a biological preparation that elicits a protective immune response in a subject to which the vaccine has been administered. Preferably, the immune response confers some beneficial, protective effect to the subject against a subsequent challenge with the infectious agent. More preferably, the immune response prevents the onset of or ameliorates at least one symptom of a disease associated with the infectious agent, or reduces the severity of at least one symptom of a disease associated with the infectious agent upon subsequent challenge.

Individual and isolated peptides comprising B- and/or T-cell epitopes and comprising the amino acid sequences as described herein (resp. characterized by SEQ ID NO 89, 90, 92-96, and 98; and by SEQ ID NO 1, 7, 8, 9, 10, 11, 12, 13, 18, 20, 21, 22, 24, 25, 26, 27, 29, 30, 31, 32, 35, 41, 42, 43, 44, 46, 49, 50, 51, 53, 54, 55, 56, 60, 61, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 91, 97, and 99) are also part of the present invention, as well as the nucleic acids encoding them. As demonstrated, said peptides are particular useful for the development of a vaccine against a species of the genus *Chlamydia*, and more specific *C. psittaci*. Hence, any combination of two or more of these peptides for producing a polyepitope vaccine and for use in developing a composition or vaccine is part of the present invention. In a specific embodiment, the polyepitope vaccine comprises or consists of a combination of two or more peptides comprising a T cell epitope as described herein. In a further embodiment, the polyepitope vaccine comprises or consists of a combination of two or more peptides comprising a B-cell epitope of the present invention.

In a further aspect of the current invention, administration of the composition as described herein, and in particular a vector comprising the peptides or nucleic acids of the invention, can overcome the inactivating (i.e. neutralizing) effects of maternal antibodies. It is well-known in the art that maternally-transmitted antibodies interfere with the efficacy of early vaccination programs in young subjects. Accordingly, the present invention provides a composition and the use thereof for effectively vaccinating a subject infected with a species of the genus *Chlamydia* that has maternal antibodies against said species. For birds, the composition can be administered according to the present invention in ovo and to hatchlings. In bird embryos, maternal antibodies are deposited in the yolk and are taken up by the embryo as the yolk is resorbed. Typically, maternal antibodies can be detected in the embryo by embryonic day 15. Accordingly, the present invention is useful in increasing the efficacy of vaccines administered after embryonic day 15, more preferably after embryonic day 17, to birds in ovo. Additionally, the methods disclosed herein may be carried out to vaccinate a young bird soon after hatch. In young chickens, maternal antibodies generally disappear by three weeks after hatch. Accordingly, in young birds, the composition of the present invention is administered within about four weeks post-hatch, preferably within about three weeks post-hatch, more preferably within about two weeks post-hatch, still more preferably, within about one week post-hatch, and most preferably within about the first three days post-hatch. Typically,

vaccination will be carried out at the time that the birds are transferred from the hatcher (usually one or two days post-hatch).

In an even further embodiment, the invention includes a prime-boost immunization or vaccination against a species of the genus *Chlamydia*. The priming can be done with the composition, peptides or nucleic acids as described herein. Equally, boosting can be done with the composition, peptides or nucleic acids as described herein.

The invention thus also relates to a method of immunizing a subject against a species of the genus *Chlamydia*, more specific *C. psittaci*, comprising administering to the subject the composition as described herein in a prime-boost regimen. In its broadest sense, the term of "prime-boost" refers to the successive administrations of two different vaccine types or immunogenic composition types having at least one epitope or immunogen in common. The priming administration is the administration of a first vaccine or composition type and may comprise one, two or more administrations. The boost administration is the administration of a second vaccine or composition type and may comprise one, two or more administrations, and, for instance, may comprise or consist essentially of annual administrations. The "boost" may be administered from about 2 weeks to about 6 months after the "priming", such as from about 2 to about 8 weeks after the priming, and advantageously from about 2 to about 6 weeks after the priming, and more advantageously, about 2, 3 or 4 weeks after the priming. In a specific embodiment, the prime and boost compositions are the same, and in particular the same peptide composition as described herein.

The present invention further relates to an in vitro method to diagnose an infection with *Chlamydia psittaci* in a subject comprising:

- contacting a sample from said subject with at least one peptide comprising a B-cell epitope of the present invention, and
- determining the presence of antibodies in said sample binding to said peptide(s).

Such methods are well-known to the skilled person and can be performed by a variety of standard procedures, such as detection of radioactivity, fluorescence, luminescence, chemiluminescence, absorbance, or by microscopy, imaging, etc.

The present invention further encompasses a diagnostic kit comprising at least one peptide comprising a B-cell epitope as described herein. Examples of diagnostic kits include but are not limited to immunoassays including immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), western blotting, immunoradiometric assay (IRMA), lateral

flow, dipstick tests, immuno histo/cyto-chemistry and other assays known to those of skill in the art. Immunoassays can be used to determine presence or absence of an antibody in a sample as well as the amount of an antibody in a sample.

A “sample” may include fluid (e.g. blood, serum, saliva, urine, cerebral spinal fluid, pleural fluid, milk, lymph, meat juice, sputum and semen), solid (e. g., stool) or tissue (e.g. cervical tissue) sections taken (isolated) from a subject.

In a particular embodiment, the peptides comprising a B-cell epitope used in the diagnostic method and kit comprises or consists of the following amino acid sequence: 1) TGTASATT (SEQ ID NO 95; immunodominant B cell epitope in VS1), 2) KGTDFFNQ (SEQ ID NO 96; serovar D epitope in VS2) and/or 3) NPTLLGKA (SEQ ID NO 94; immunodominant B cell epitope in VS4), or a variant thereof. Even more particular, the peptide has a length of 5 to 30 amino acids.

The present invention is illustrated by the following Examples, which should not be understood to limit the scope of the invention to the specific embodiments therein.

Examples

Example 1: *C. psittaci* subunit vaccination experiment

We performed B- and T cell epitope mapping of the *C. psittaci* ‘major outer membrane protein’ (MOMP) and used B- and T cell epitopes to create a *C. psittaci* subunit (polyepitope) vaccine, which was validated in a pre-clinical trial in specific pathogen free (SPF) chickens. *C. psittaci* strain 92/1293, isolated from a severe outbreak of respiratory disease in a commercial broiler turkey farm in the Netherlands, was used (Vanrompay et al., 1993). The strain was isolated from a pooled homogenate of the lungs, the cloacae and the spleens of diseased turkeys and was characterized as serovar D and genotype D (Geens et al., 2005). Bacteria were grown in Buffalo Green Monkey (BGM) cells as previously described (Vanrompay et al., 1992) and the titration was performed by the method of Spearman and Kaerber (Mayr et al., 1974).

1. B cell epitope mapping

For B cell epitope identification, overlapping synthetic peptides of 8 amino acids (7 amino acids overlap) of the variable domains I to IV of the MOMP sequence were coupled on pins

via an extra C-terminal cysteine (Pin-peptides, Pepscan Systems, Lelystad, The Netherlands). A total amount of 100 nmol peptide was coupled to each pin.

The results of the pin-peptide ELISAs for B cell epitope mapping of the MOMP of *C. psittaci* serovar D strain 92/1293, a highly virulent genotype D strain infecting poultry, are presented in Fig. 3. When using the serovar-D specific MAb at a dilution of 1/100 (Fig. 3A), peptide 23 gave the highest signal. At a MAb dilution of 1/600 peptide 23 still gave a high signal while the signal created by the other peptides weakened significantly. Pin-peptide 23 (KGTFDFNNQ) is most likely the serovar-D specific epitope of *C. psittaci* genotype D and can be found in the variable sequence region 2 (VS2) of MOMP.

When using serum of SPF turkeys immunized with recombinant MOMP of strain 92/1293, peptide 23 gave a weak signal (Fig. 3B), which indicates that peptide 23 is not predominantly recognized by the immunized animals. However, a very strong signal could be obtained for peptide 12 (TGTASATT) located in VS1 and peptide 43 (NPTLLGKA) located in VS4. The same signal pattern was also obtained when using serum of naturally infected turkeys. Accordingly, the peptides TGTASATT, NPTLLGKA and KGTFDFNNQ are immunodominant (strongly recognized by the immune system as shown in Figure 3) B-cell epitopes and are useful for vaccine development.

Moreover the B-cell epitopes 12 and 43 induce sero-neutralizing antibodies by immunizing SPF chickens with peptide 12 or 43 coupled to the carrier protein KLH (keyhole limpet hemocyanin).

The identified B cell epitopes TGTASATT and NPTLLGKA can be used in an antibody ELISA for detecting *C. psittaci* antibodies in both humans and animals. Immunodominant B cell epitopes can be used as pin-peptides in a highly sensitive antibody ELISA. The serovar specific epitope KGTFDFNNQ can be used as pin-peptide in a serovar D-specific antibody ELISA.

2. T cell epitope mapping

For T cell epitope identification, overlapping synthetic peptides of 15 amino acids (14 amino acids overlap) of the complete MOMP sequence were produced (Pep-T-Topes, Pepscan Systems) in 96-well flat bottom tissue culture plates (Greiner Bio-one, Wemmel, Belgium) with an amount of 1-2 mg peptide per well.

Pep-T-Topes were used to identify the T cell epitopes on the MOMP of *C. psittaci* serovar D strain 92/1293. T-cell epitope mapping was based on: a) lymphocyte proliferation assays, b) flow cytometry on proliferating cells using CD4 and CD8 cell surface markers, c) an IFN- γ

ELISA on supernatant of proliferating cells and d) an IL-6 bioassay on supernatant of proliferating cells (Lynagh et al., 2000; Zubiaga et al., 1990). Table 1 contains the complete results of B-and T-epitope mapping.

Peptides eliciting one or more of following characteristics are categorized as suitable for vaccine design:

- a) Counts per minute (Cpm) in proliferation assay > 10000,
- b) %CD4 $\geq$ 17,5,
- c) %CD8 > 14,2,
- d) IFN- γ > 9 pg/ml, and
- e) IL-6 > 1,9 pg/ml.

Peptides comprising T-cell epitopes suitable for vaccine design correspond to SEQ ID NO 1, 7, 8, 9, 10, 11, 12, 13, 18, 20, 21, 22, 24, 25, 26, 27, 29, 30, 31, 32, 35, 41, 42, 43, 44, 46, 49, 50, 51, 53, 54, 55, 56, 60, 61, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, and 91. From these, peptides especially useful to include in the composition of the present invention are the following:

- Peptides comprising a CTL (CD8+) epitope correspond to SEQ ID NO 20, 26, 27, 42, 43, 61, 73, 75, and 80.
- Peptides comprising a CD4+ Th1 cell epitope correspond to SEQ ID NO 1, 10-13, 18, 20, 25, 27, 29, 31-32, 35, 42, 46, 51, 53-56, 61, 64-67, 69-71, 73, 75, 79-81, and 87.
- Peptides comprising a CD4+ Th2 cell epitope correspond to SEQ ID NO 7, 8, 43, 76, 77, 85, and 91.

From these peptides, a first selection was made to design a polyepitope vaccine.

Many CD4 Th1 epitopes were present on the MOMP and they were found in combination with CD8 epitopes. Especially peptide 281-295 contains a very strong CD4 Th1-CD8 epitope (108.088 cpm). Moreover, these epitopes are located in a large cluster of multiple CD4 Th1 epitopes, one additional CD8 epitope and two CD4 Th2 epitopes present on a peptide, covering amino acids 257 to 296.

Table 1. Peptides harbouring B- and T-cell epitopes of *Chlamydia psittaci* genotype D strain 92/1293

SEQ ID NO	position	Sequence	TC (cpm) ¹	% CD4	% CD8	IFN- γ (pg/ml)	IL-6 (pg/ml)	Epitope
1	17-31	ALSLQALPVGNPAEP	11093	13,6	0,4	9	0	CD4 Th1
2	18-32	LSLQALPVGNPAEPS	6393	1,7	0,8	9	0	CD4 Th1
3	25-39	VGNPAEPSLLIDGTM	4545	9,4	0,7	9	0	/
4	26-40	GNPAEPSLLIDGTMW	2027	4,9	1,0	0	0	/
5	29-43	AEPSLLIDGTMWEGA	4851	10,8	2,1	0	1,9	/
6	30-44	EPSLLIDGTMWEGAS	7421	6,6	1,6	0	1,9	CD4 Th2
7	35-49	IDGTMWEGASGDPCD	14032	20,5	3,8	0	3,9	CD4 Th2
8	36-50	DGTMWEGASGDPCDP	9462	1,8	1,0	0	3,9	CD4 Th2
9	37-51	GTMWEGASGDPCDPC	15074	3,6	0,9	0	0	/
10	40-54	WEGASGDPCDPCATW	14332	1,6	1,0	9	0	CD4 Th1
11	41-55	EGASGDPCDPCATWC	13289	1,4	1,0	9	0	CD4 Th1
12	42-56	GASGDPCDPCATWCD	14731	1,6	1,0	9	0	CD4 Th1
13	43-57	ASGDPCDPCATWCDA	16929	4,0	1,2	9	0	CD4 Th1
14	44-58	SGDPCDPCATWCDAI	3385	0,6	0,2	0	0	/
15	46-60	DPCDPCATWCDAISI	5042	2,6	1,3	0	1,9	CD4 Th2
16	47-61	PCDPCATWCDAISIR	5856	5,9	1,0	0	0	
17	48-62	CDPCATWCDAISIRA	2252	2,2	1,0	0	0	/

18	49-63	DPCATWCDAISIRAG	14852	8,4	0,8	9	0		CD4 Th1
19	50-64	PCATWCDAISIRAGY	8720	6,9	1,8	0	0		/
20	53-67	TWCDAISIRAGYYGD	35485	13,6	6,9	9	0		CD4 Th1 CD8
21	54-68	WCDAISIRAGYYGDY	30171	17,3	1,5	0	0		CD4
22	55-69	CDAISIRAGYYGDYV	3668	37,0	7,0	0	0		/
23	58-72	ISIRAGYYGDYVFDR	3684	16,4	1,9	0	0		/
24	59-73	SIRAGYYGDYVFDRV	11032	21,5	2,1	0	0		CD4
25	60-74	IRAGYYGDYVFDRVL	18411	12,1	0,9	9	0		CD4 Th1
26	61-75	RAGYYGDYVFDRVLK	12444	21,4	4,6	0	0		CD4 + CD8
27	66-80	GDYVFDRVLKVDVNK	5363	24,0	7,8	9	0		CD4 Th1 CD8
28	67-81	DYVFDRVLKVDVNKT	5366	4,1	1,2	9	0		CD4 Th1
29	71-85	DRVLKVDVNKTFSGM	9311	5,7	0,9	18	0		CD4 Th1
30	72-86	RVLKVDVNKTFSGMA	4741	19,4	2,1	0	0		/
31	73-87	VLKVDVNKTFSGMAK	27420	8,0	1,1	9	0		CD4 Th1
32	85-99	MAKSPTEATGTASAT ²	80.468	25,1	2,0	32	0		CD4 Th1 B-cell epitope
33	91-105	EATGTASATTTAVDR	3433	1,7	0,8	9	0		B-cell epitope
34	95-109	TASATTTAVDRTNLA	8278	2,6	0,7	0	0		CD4
35	97-111	SATTTAVDRTNLAYG	60.941	16,4	2,9	18	0		CD4 Th1
36	98-112	ATTTAVDRTNLAYGK	2241	2,7	1,2	9	0		/

37	107-121	NLAYGKHLQDAEWFT	2532	2,6	0,6	0	0	/
38	109-123	AYGKHLQDAEWFTNA	6018	3,9	1,2	9	0	CD4 Th1
39	111-125	GKHLQDAEWFTNAAF	3621	12,2	3,4	9	0	/
40	112-126	KHLQDAEWFTNA AFL	2513	4,1	1,1	0	0	/
41	113-127	HLQDAEWFTNA AFLA	2546	19,7	5,8	9	0	/
42	119-133	WFTNA AFLALNIWDR	25.067	18,7	12,1	9	0	CD4 Th1 CD8
43	131-145	WDRFDIFCTLGASNG	5775	58,7	14,9	0	1,9	CD4 Th2 CD8
44	142-156	ASNGYFKASSAAFNL	2009	18,7	4,0	0	0	/
45	154-168	FN LGVLIGLKGTD FN ³	1129	10,6	2,1	0	0	B-cell epitope
46	166-180	DFNNQLPNVAITQGV	5683	18,3	2,3	9	0	CD4 Th1 B-cell epitope
47	167-181	FN NQLPNVAITQGVV	1321	2,3	0,9	9	0	/
48	168-182	NNQLPNVAITQGVVE	1262	15,9	6,1	9	0	/
49	184-198	YTDTTFSWSVGARGA	2811	44,1	18,3	9	0	/
50	195-209	ARGALWECGCATLGA	7924	18,3	6,1	0	0	CD4
51	196-210	RGALWECGCATLGAE	21.981	7,5	2,6	9	0	CD4 Th1
52	197-211	GALWECGCATLGA EF	4416	5,3	0,1	0	0	/
53	201-215	ECGCATLGAEFQY AQ	15.883	1,6	0,9	9	0	CD4 Th1
54	202-216	CGCATLGAEFQY AQ S	23.344	2,1	0,4	18	0	CD4 Th1

55	203-217	GCATLGAEFQYAQSN	10.217	4,5	2,8	18	0	CD4 Th1
56	204-218	CATLGAEFQYAQSNP	6924	21,9	4,3	18	0	CD4 Th1
57	205-219	ATLGAEFQYAQSNPK	6533	7,1	1,3	9	0	CD4 Th1
58	206-220	TLGAEFQYAQSNPKI	4225	12,4	1,7	9	0	/
59	207-221	LGAEFQYAQSNPKIE	1218	10,7	3,6	9	0	/
60	208-222	GAEFQYAQSNPKIEM	2142	3,1	1,1	18	0	/
61	214-228	AQSNPKIEMMLNVTSS	21.643	28,9	7,3	18	0	CD4 Th1 CD8
62	221-235	EMLNVTSSPAQFVIH	7037	16,0	4,4	0	1,9	CD4 Th2
63	222-236	MLNVTSSPAQFVIHK	2178	24,2	5,8	18	0	/
64	233-247	VIHKPRGYKGTGSNF	44.429	23,3	0,8	9	0	CD4 Th1
65	244-258	GSNFPLPIDAGTEAA	25.253	4,8	0,6	9	0	CD4 Th1
66	245-259	SNFPLPIDAGTEAAT	39.106	5,4	0,6	9	0	CD4 Th1
67	246-260	NFPLPIDAGTEAATD	13.698	17,5	0,5	9	0	CD4 Th1
68	251-265	IDAGTEAAATDTKSAT	15.894	8,1	1,2	0	0	CD4
69	252-266	DAGTEAAATDTKSATL	10.513	77,7	5,7	9	0	CD4 Th1
70	257-271	AATDTKSATLKYHEW	39.489	8,3	0,4	9	0	CD4 Th1
71	262-276	KSATLKYHEWQVGLA	19.151	13,2	3,0	32	0	CD4 Th1
72	263-278	SATLKYHEWQVGLAL	2841	5,4	0,9	18	0	CD4 Th1
73	264-279	ATLKYHEWQVGLALS	20.815	48,8	7,1	18	0	CD4 Th1 CD8
74	266-280	LKYHEWQVGLALS	3013	61,0	14,2	9	0	/

75	267-281	KYHEWQVGLALSYRL	13.516	43,5	20,4	9	0	CD4 Th1 CD8
76	268-282	YHEWQVGLALSYRLN	20.591	20,6	1,4	0	1,9	CD4 Th2
77	269-283	HEWQVGLALSYRLNM	62.709	39,4	5,8	0	3,9	CD4 Th2
78	279-293	YRLNMLVPYIGVNWS	5510	61,7	5,4	0	1,9	CD4
79	280-294	RLNMLVPYIGVNWSR	41.321	60,4	3,6	9	0	CD4 Th1
80	281-295	LNMLVPYIGVNWSRA	108.088	65,8	20,2	9	0	CD4 Th1 CD8
81	282-296	NMLVPYIGVNWSRAT	28.136	30,9	0,9	9	0	CD4 Th1
82	287-302	YIGVNWSRATFDADT	14.406	6,8	0,6	0	0	CD4
83	291-305	NWSRATFDADTIRIA	2483	16,0	2,8	0	0	/
84	291-306	WSRATFDADTIRIAQ	21.434	26,4	3,9	0	0	CD4
85	293-307	SRATFDADTIRIAQP	41.778	8,6	1,2	0	1,9	CD4 Th2
86	295-309	ATFDADTIRIAQPKL	2539	18,5	1,7	0	0	/
87	299-313	ADTIRIAQPKLATAV	10.759	12,0	2,2	9	0	CD4 Th1
88	304-318	IAQPKLATAVLDLTT	1829	10,8	3,1	0	0	/
89	305-319	AQPKLATAVLDLTTW	6541	7,7	0,5	0	0	Flanking the B- cell epitope NPTLLGKA
90	311-325	TAVLDLTTWNPTLLG ⁴	5087	6,4	0,6	0	1,9	Part of the B-cell epitope

3. *C. psittaci* vaccine

Peptides including the identified B and T cell epitopes of the MOMP of *C. psittaci* strain 92/1293 were synthetically designed.

5 4. *Vaccination trial 1: vaccination of SPF chickens*

Experiments were performed in negative pressure isolators (IM 1500, Montair Sevenum, the Netherlands). The experimental design was evaluated and approved by the Ethical Commission for Animal Experiments of Ghent University. To evaluate the polyepitope vaccine, 42 specific pathogen free (SPF) chickens (Löhman, Germany) were divided into 6 groups of 7 animals each.

10 The vaccination scheme and the experimental set-up are presented in Table 3 and 4. The animals were vaccinated by aerosol (CirrusTM nebulizer; 5 µm aerosol particle size; Laméris, Aartselaar, Belgium) providing a vaccine dose of 500 µg of each synthetic peptide/animal. The control animals each received 60 µg of ISCOMS by aerosol. Primo vaccination was performed in 7 day-old chickens of groups 1 to 6 (Table 3). At the age of 4 weeks, animals of groups 1 to 4 were

15 aerogenically infected using the CirrusTM nebulizer, while those of groups 5 and 6 received a booster vaccination. At the age of 7 weeks, all animals of groups 1 to 4 were euthanized, while those of groups 5 and (age 6 weeks) were aerogenically infected using the CirrusTM nebulizer. The experimental infection dose in each isolator was 10⁶ TCID₅₀ of strain *C. psittaci* 92/1293. Animals of groups 5 and 6 were euthanized at 21 days post infection (p.i.).

20

Peptide 1: EPSLLIDGTMWEGASGDPCDPC (SEQ ID NO 97)

Peptide 2: TGTASATT (SEQ ID NO 95)

Peptide 3: KGTDFNNQ (SEQ ID NO 96)

Peptide 4: AQPKLATAVLDLTTWNPTLLGKATTVDGTNTYSDFL (SEQ ID NO 98)

25 Peptide 5: AATDTKSATLKYHEWQVGLALSYRLNMLVPYIGVNWSRATFDADT (SEQ ID NO 99)

30

Table 3. Vaccination scheme.

Group (n)	Name	Primo vaccination (day 7)	Dose (µg)	Booster vaccination (day 21)	Dose (µg)
1 (7)	Iscoms (1 x)	Iscoms	60	none	60
2 (7)	B (1 x)	Peptides 1, 2, 3 and 4	500	none	500
3 (7)	T (1 x)	Peptide 5	500	none	500
4 (7)	B + T (1 x)	Peptides 1, 2, 3, 4 and 5	500	none	500
5 (7)	Iscoms (2 x)	Iscoms	60	Iscoms	60
6 (8)	B + T (2 x)	Peptides 1, 2, 3, 4 and 5	500	Peptides 1, 2, 3, 4 and 5	500

Table 4. Timing of vaccination, challenge and euthanasia for groups 1 to 6.

Group	Primo vaccination (age in weeks)	Booster vaccination (age in weeks)	Challenge (age in weeks)	Euthanasia (age in weeks)
1	1	/	4	7
2	1	/	4	7
3	1	/	4	7
4	1	/	4	7
5	1	4	6	9
6	1	4	6	9

5 4.1. Monitoring and sampling

Clinical signs were scored daily until necropsy. Pharyngeal and cloacal excretion of *C. psittaci* was monitored on day 1 of the experiment and on every other day starting at 4 days post infection (p.i.) until necropsy at 21 days p.i., using rayon-tipped, aluminium shafted swabs (Colpan; Fiers, Kuurne, Belgium) provided with *C. psittaci* transport medium (sucrose 74,6 g/l (Acros Organics, Geel, Belgium); KH_2PO_4 5,1 g/l and K_2HPO_4 1,2 g/l (Sigma); L-glutamic acid mono potassium salt 0,9 g/l (Invitrogen, Merelbeke, Belgium) and fetal calf serum 10% v/v (Greiner, Wommel, Belgium); gentamycin 50 µg/ml (Invitrogen); vancomycin 100 µg/ml and streptomycin 100 µg/ml (Soenen, Merelbeke, Belgium); nystatin 25000 U/ml (Sigma) pH 7). Swabs were stored at -80°C until processed.

Blood samples for the detection of MOMP-specific serum antibody titres were collected prior to each vaccination, immediately prior to the experimental infection and at euthanasia at 21 days p.i.

The samples were stored overnight at room temperature, centrifuged (300×g, 10 min, 4 °C) and afterwards serum was collected. The serum was pretreated with kaolin to remove background activity (Novak et al., 1993) and stored at -80 °C.

At the time of euthanasia, 21 days p.i., proliferative responses of peripheral blood lymphocytes and spleen lymphocytes were determined by use of a T cell proliferation assay. Additionally, the amount of immune cells in blood and spleen was determined by flow cytometry. At euthanasia, all chickens were examined for gross lesions. The scoring system for gross pathology is presented in Table 5. Cryostat tissue sections of conchae, the conjunctivae, the trachea, the lungs, thoracic and abdominal airsacs, the pericardium, liver and spleen were prepared for the presence of chlamydial antigen.

Table 5. Scoring system for *C. psittaci* gross pathology present in euthanized chickens.

Tissue	Score 1*	Score 2	Score 3
Conjunctivae	Slightly congested	Severely congested	Petechiae
Conchae	Slightly congested	Severely congested	Necrosis
Trachea	Slightly congested	Moderately congested	Severely congested
Lungs	Slightly congested	Severely congested	Severely congested + grey inflam. foci
Thoracic air sacs	Diffuse opacity	Focal fibrin deposits	Severe fibrinous airsacculitis
Abdominal air sacs	Diffuse opacity	Few fibrin deposits	Severe fibrinous airsacculitis
Pericardium	Serous pericarditis	Sero-fibrinous pericarditis	Fibrinous adhesive pericarditis
Spleen	Slightly enlarged	Moderately enlarged	Severely enlarged & petechiae
Liver	Slightly enlarged	Moderately enlarged	Severely enlarged

* score 0 = no gross pathology.

4.2. Clinical signs

Clinical signs could be observed in all animals of the control groups (group 1 and 5) starting from day 4 p.i. until the end of the experiment on day 21 p.i. All control chickens appeared lethargic

and showed anorexia, conjunctivitis, rhinitis, respiratory distress and diarrhea. At first, the main symptoms were lethargia, anorexia and conjunctivitis in all. Sometimes, 6 of 7 (85%) animals also shed watery droppings. From day 8 p.i. until day 10 p.i. rhinitis appeared in all and from day 11 p.i. until day 21 p.i., all animals intermittently showed dyspnea. At euthanasia, 4 of 7 (57%)
5 had large amounts of mucus in their beak.

Clinical signs in group 2 first appeared at day 4 p.i., with 3 of 7 (43%) animals showing rhinitis and conjunctivitis. From day 9 p.i. conjunctivitis and rhinitis were observed in all. The number of animals with conjunctivitis and rhinitis gradually declined towards day 17 p.i. At that time all chickens of group 2 appeared healthy.

10 On day 4 p.i., 4 animals (67 %) of groups 3 and 4 showed conjunctivitis. One day later conjunctivitis started to diminish in those four animals but 3 of them (50 %) got rhinitis for the following four (group 3) or two (group 4) days. On day 10 and 8 p.i., chickens of groups 3 and 4, respectively, showed healthy.

15 All vaccinated animals of group 6 remained very active after the experimental infection and they were eating well. They showed no clinical symptoms.
Thus, regarding the clinical signs, protection was best in group 6, followed by group 4, 3 and 2, respectively.

20 4.3. Macroscopic lesions

The results of the pathological examination at euthanasia are presented in Figure 5. Mean scores per group are presented $\pm$ SD. Groups 2, 3 and 4 were statistically compared to the control group 1. Group 6 was statistically compared to the control group 5.

25 First of all, we need to discuss the observations for the spleen as they are not presented in Figure 5. The spleen of all control chickens was moderately congested and 2 spleens of chickens of group 1 and one spleen of a chicken of group 5 showed petechiae. Slight to moderate congestion was also observed in spleens of chickens of groups 2 and 3. The spleen of one chicken in each of the latter groups also showed petechiae. The spleen of the chickens of group 4 and 6 was
30 moderately to severely enlarged, but instead of congested, the spleens appeared swollen with

macroscopically visible white pulpa, indicative for immunostimulation. The latter was more prominent in chickens of group 6 as compared to group 4.

Control chickens of group 1 showed gross pathology in all examined tissues but one, namely the trachea. In group 2, gross pathology was observed in 6 of 10 (60%) examined tissues. In group 3, gross pathology was observed in 9 of 10 (90%) examined tissues. However, the lesions in the trachea of some chickens were most probably due to the euthanasia. Thus, in group 3, gross pathology was observed in 8 of 10 (90%) examined tissues.

Overall, group 4 was significantly better protected than the control group 1. Protection in group 4 was also better than for groups 2 and 3, as gross pathology was only observed in 1 of 10 (10%) examined tissues, namely the lungs. Gross pathology was observed in the lung of 2 of 7 animals of group 4.

Control chickens of group 5 showed gross pathology in all examined tissues but one, namely the trachea. In group 6, gross pathology was only observed in the lung in 1 of 8 animals.

Liver lesions were only observed in groups 1, 2, 3 and 5 indicative for septicaemia at the time of euthanasia.

Thus, regarding the autopsy results, protection was best in groups 6 and 4.

4.4. *Chlamydia* replication and excretion

Cryostat tissue sections (5 µm) of the conchae, the conjunctivae, the trachea, the lungs, thoracic and abdominal airsacs, the pericardium, liver and spleen were examined for *C. psittaci* replication by the IMAGENTM immunofluorescence staining (IMAGENTM CHLAMYDIA, Oxoid, Drongen, Belgium), as previously described (Vanrompay et al., 1994a). *C. psittaci* positive cells were enumerated in five randomly selected microscopic fields (600x, Nikon Eclipse TE2000-E, Japan) and scored between 0 and 5: score 0 indicated no *C. psittaci* present; score 1 was given when a mean of 1-5 elementary bodies (infectious, non-metabolic morphological form) was present; score 2, 3, 4 and 5 were given when a mean of 1-5, 6-10, 10-20 and > 20 inclusion (actively replicating organisms) positive cells was present.

Swabs were examined for *C. psittaci* excretion by bacterial culture in Buffalo Green Monkey (BGM) cells (Vanrompay et al., 1992) and subsequent chlamydial identification using the

IMAGENTM direct immunofluorescence assay (Vanrompay et al., 1994b). The presence of *C. psittaci* was scored as for bacterial replication in different tissues. Swabs taken at day 1 of the experiment were negative for *C. psittaci*. Culture results for swabs taken at 21 days post infection (euthanasia) are presented in Table 6. The results for the remaining swabs are presented in Table 22.

Table 6. Pharyngeal and cloacal excretion of *C. psittaci* at euthanasia, 21 days p.i.

Pharyngeal excretion						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 21 p.i	1,71 ± 0,39	1.0 ± 0,40	0,5 ± 0	0,5 ± 0	1,78 ± 0,26	0,5 ± 0
Cloacal excretion						
Day 21 p.i.	2.0 ± 0	0,71 ± 0,39	0,5 ± 0	0,5 ± 0	1,92 ± 1,01	0,5 ± 0

At euthanasia, *C. psittaci* pharyngeal excretion was significantly higher in groups 1 and 2, as compared to groups 3 and 4. At euthanasia, pharyngeal *C. psittaci* excretion was significantly higher for group 5 than for group 6. The same was the case for the cloacal excretion. Thus, regarding *C. psittaci* excretion at 21 days p.i., protection was equally best in groups 3, 4 and 6.

4.5. Antibody responses

The serum samples (Figure 6) and mucosal swabs (Figure 7) of the chickens were analyzed by an enzyme-linked immunosorbent assay (ELISA). The total IgG(H+L) antibody titers were determined as previously described, using the recombinant MOMP, hereinafter called rMOMP, antigen to coat the plates (Verminnen et al., 2006). Two-fold dilutions, starting at 1/40 for sera and 1/20 for mucosal swabs and taking into account the dilution made by the kaolin treatment, were incubated into the wells to react with the rMOMP coating. Detection MOMP-specific isotypes still needs to be done 1/500 dilutions of cross-reactive anti-chicken IgG-, IgM- and IgA specific peroxidase-conjugated polyclonal antibodies (Bethyl Laboratories, Inc., Montgomery, USA). After the addition of the substrate H₂O₂ and the chromogen ABTS, the maximum absorbance at 405 nm could be determined. Samples were considered positive if the absorbance exceeded the cut-off value, calculated as the mean of the negative control absorbencies plus twice

the standard deviation. Titers were presented as the reciprocal of the highest serum dilution with an absorbance above the cut-off value. As a positive control, serum obtained from previous vaccination experiments was used.

5 First the comparison of groups 1 to 4. On day -14, the mean antibody titer of group 1 was significantly lower than the mean antibody titers of groups 3 and 4. At that time, the mean antibody titer for group 1 was statistically the same as for group 2. However, mucosal primo immunization did not result in significant higher serum antibody titers at the day of infection (day -1). At day -1, mean serum antibody titers in groups 2, 3 and 4 were statistically the same as for
10 both control group 1. Infection of groups 1, 2, 3 and 4 resulted in augmenting serum antibody titers in the control group 1 and in the better-protected group 4. At 22 days post infection, the mean serum antibody titer for group 4 was significantly higher than for the control group 1 and than for groups 2 and 3.

Secondly, the comparison of groups 5 and 6. Again, on day -14, the mean serum antibody titer of
15 group 5 was significantly lower than the mean antibody titer of group 6. Again, mucosal primo immunization did not result in significant higher serum antibody titers at the day of infection (day -1). At day -1, the mean serum antibody titer in group 6 was statistically the same as for the control group 5. However, one week prior to the infection, the mean serum antibody titer in group 6 was significantly higher than for the control group 5. The same was true at two days and
20 one week after the experimental infection. Thereafter, serum antibodies in the best-protected group 6 declined rapidly while those in the unprotected control group 5 rapidly augmented till 14 days post infection.

Most relevant information is the significant increase of mean mucosal antibody titers following
25 mucosal primo vaccination in the better-protected groups 4 and 6 (day -1), as compared to the control groups 1 and 5 and the immunized groups 2 and 3. Chickens in groups 4 and 6 received the same vaccine. Infection of groups 1, 2, 3 and 4 at day -1 resulted in augmenting mean mucosal antibody titers in the unprotected group 1 and the less protected groups 2 and 3. The mean mucosal antibody titers in the better-protected group 4 generally declined following
30 infection. Booster vaccination in group 6 had no visible effect on the mean mucosal antibody titer. Infection of groups 5 and 6, first resulted in a rapidly augmenting and a rapidly declining

mean mucosal antibody titer in the unprotected group 5 and the best protected group 6, respectively. From 7 days p.i. onwards mean mucosal antibody titers in the best protected group 6 continued to rise till 21 days p.i., while those in the unprotected group 5 declined and even disappeared at 14 days p.i.

5

4.6. Lymphocyte proliferative responses

Peripheral blood leukocytes (PBL) were isolated from heparinized blood samples obtained by venepuncture (*V. ulnaris*) and from the spleen from each chicken, at 21 days p.i. at euthanasia. Lymphocyte proliferative tests were performed as previously described (Vanrompay et al., 10 1999b). Briefly, non-adherent cells were grown in duplicate in 96-well tissue culture plates at 10^6 cells in 150 μ l of DMEM (Invitrogen) supplemented with 20% heat-inactivated fetal calf serum (Greiner), 1% nonessential amino acids, 1% sodium pyruvate, 1% L-glutamine, 1% gentamycine and 0,0001% β -mercaptoethanol (all Invitrogen). For antigen proliferation, 20 μ g of recombinant MOMP was added to individual wells. Negative and positive controls included cells stimulated 15 with either plain medium or with 10 μ g concanavalin A (Con A), respectively. Cells were incubated at 39,5 °C in a humidified incubator with 5% CO₂. Con A or antigen-induced proliferation was measured by incorporation of ³H-thymidine (1 μ Ci/well) during at last 16 h of culture, at days 2 (ConA) and 6, respectively. Cultures were harvested onto glass fiber filter strips with a cell harvester (Skatron, Liers, Norway). The radioactivity incorporated into the DNA was 20 measured with a β -scintillation counter (Perkin-Elmer, Brussels, Belgium). The stimulation index (SI) was defined as the ratio of counts per minute (cpm) of stimulated cultures on medium-only cultures. Results are presented in Table 7.

4.7. Statistical analysis

25 The non-parametric Kruskal-Wallis and Mann-Whitney tests were employed for statistical analyses. Results were considered to be significantly different at the level of $p < 0,05$.

Table 7. Mean stimulation index $\pm$ standard error mean.

	Blood lymphocytes		Spleen lymphocytes	
	Inactivated bacteria	Recombinant MOMP of <i>C. psittaci</i>	Inactivated bacteria	Recombinant MOMP of <i>C. psittaci</i>
Group 1	1.48 $\pm$ 0.24	1.72 $\pm$ 0.27	1.02 $\pm$ 0.10	1.54 $\pm$ 0.46
Group 2	3.92 $\pm$ 1.66	1.58 $\pm$ 0.38	1.76 $\pm$ 0.09	1.17 $\pm$ 0.70
Group 3	4.2 $\pm$ 2.19	2.09 $\pm$ 0.97	1.67 $\pm$ 0.98	1.34 $\pm$ 0.13
Group 4	7.9 $\pm$ 0.0	8.37 $\pm$ 2.74	NT	3.85 $\pm$ 0.56
Group 5	1.22 $\pm$ 0.19	1.76 $\pm$ 0.25	1.32 $\pm$ 0.41	0.92 $\pm$ 0.25
Group 6	5.03 $\pm$ 1.41	3.13 $\pm$ 0.74	17.6 $\pm$ 6.41	17.9 $\pm$ 6.89

For the primo vaccinated groups 1 to 4, protection was correlated with a significantly higher stimulation index for *in vitro* restimulation of blood lymphocytes with inactivated *C. psittaci* whole organisms as well as with recombinant MOMP of *C. psittaci*. For the primo vaccinated groups 1 to 4, protection was correlated with a significantly higher stimulation index for *in vitro* restimulation of spleen lymphocytes with recombinant MOMP of *C. psittaci*. Unfortunately, we could not test inactivated bacteria for group 4.

For groups 5 and 6, protection was correlated with a significantly higher stimulation index for *in vitro* restimulation of blood and spleen lymphocytes with inactivated *C. psittaci* whole organisms as well as with recombinant MOMP of *C. psittaci*.

Protection was correlated with higher CD4/CD8 ratios for peripheral blood lymphocytes at any time point post infection.

Conclusion vaccination trial 1

Groups 4 and 6 receiving a combination of B and T cell epitopes were significantly protected against clinical signs, gross pathology and bacterial excretion. Regarding clinical signs, groups 6 was better protected than group 4. Protection was correlated with superior B cell responses upon immunisation (higher mucosal antibody titers post primo vaccination and higher serum antibody titers following booster vaccination), with high proliferative responses of T cells at euthanasia and with a higher CD4/CD8 ratio for peripheral blood lymphocytes at any time point post infection.

5. Vaccination trial 2: vaccination of SPF chickens

From the peptides described herein, a second selection of B-cell, CD4⁺ Th1, CD4⁺ Th2 and CD8 T-cell epitopes was made to design a polyepitope vaccine.

5

Table 8. Peptides used in the second vaccination experiment

Peptide ID	SEQ ID NO	position	Sequence	Epitopes
X	20	53-67	TWCDAISIRAGYYGD	CD4Th1 + CD8
Y	62	221-235	EMLNVTSSPAQFVIH	CD4Th2
Z	96	163-170	KGTDFFNNQ	B cell

Experiments were performed in negative pressure isolators (IM 1500, Montair Sevenum, the Netherlands). The experimental design was evaluated and approved by the Ethical Commission for Animal Experiments of Ghent University. To evaluate the vaccine, 18 specific pathogen free (SPF) chickens (Löhman, Cuxhaven, Germany) were divided into three groups of 6 animals. The vaccination scheme is presented in Table 9. Group 1 received 500 µg of each peptide X, Y and Z per animal. In group 2, 3 chickens received 500 µg of peptide X (subgroup 2a), while the other 3 chickens received 500 µg of peptide Y and 500 µg of peptide Z (subgroup 2b). The vaccine was administered as an aerosol using the CirrusTM nebulizer (2-5 µm aerosol particle size; Laméris, Aartselaar, Belgium). The control animals of group 3 received no vaccine. Vaccination was performed at the age of 6 weeks. At the age of 8 weeks, all animals were aerogenically infected using a CirrusTM nebulizer (2-5 µm aerosol particle size; Laméris, Aartselaar, Belgium). The experimental infection dose in each isolator was 10⁶ TCID₅₀. Animals were euthanized at 10 days post infection (dpi).

Table 9. Vaccination scheme.

Group	n	Vaccination (week 6)	Epitope	Dose
1	6	Peptides X, Y and Z	B + CD4Th2 CD8 + CD4Th1	500 µg/peptide/animal
2a	3	Peptide X	CD8 + CD4Th1	500 µg/animal

2b	3	Peptides Y + Z	B + CD4Th2	500 µg/peptide/animal
3	6	Non-vaccinated control	/	/

5.1. Monitoring and sampling

Clinical signs were monitored daily until necropsy at 10 dpi.

- 5 Blood samples for the detection of MOMP-specific serum antibody titres were collected prior to vaccination, at the day of challenge and at euthanasia at 10 dpi. Blood samples were stored overnight at room temperature, centrifuged (325×g, 10 min, 4 °C) and afterwards serum was collected and stored at -20 °C.

- 10 At the time of euthanasia, 10 days p.i., all turkeys were examined for gross lesions. Impression smears (cytology) of the lungs and spleen were examined for the presence of chlamydial antigen.

All animals of the control group 3 showed conjunctivitis, rhinitis and moderate dyspnoea. Symptoms were most severe from 8 to 10 dpi. No clinical signs were observed in the vaccinated groups 1 or 2.

- 15 The control animals showed severe congestion of the conjunctiva, sometimes only unilaterally, severe congestion of the conchae, moderate congestion of the trachea, bilateral congestion of the lungs with few grey foci surrounded by a hyperaemic zone (pneumonia), opacity of the airsacs and few fibrin clots in the abdominal airsac, mostly unilateral and hepatosplenomegaly. Group 1, immunized with peptides X, Y and Z showed no macroscopic lesions. Group 2a, immunized
20 with peptide X, showed slight (2 of 3 chickens) to strong (1 of 3 chickens) congestion of the lungs, slight congestion of the serosa of the small intestine (especially the duodenum) and in one of 3 chickens serous pericarditis was observed. The animals of group 2b, immunized with peptides Y and Z only showed slight (1 of 3 chickens) to strong (2 of 3 chickens) congestion of the lungs. No other lesions were observed.

25

5.2. Chlamydia replication in lungs and spleen

Impression smears (cytology) of the lungs and spleen were examined for *C. psittaci* replication by the IMAGENTM immunofluorescence staining (IMAGENTM CHLAMYDIA, Oxoid, Drongen, Belgium), as previously described (Vanrompay et al., 1992). *C. psittaci* positive cells were

enumerated in five randomly selected microscopic fields (400x, Nikon Eclipse TE2000-E, Japan) and scored between 0 and 5: score 0 indicated no *C. psittaci* present; score 1 was given when a mean of 1-5 elementary bodies (infectious, non-metabolic morphological form) was present; score 2, 3, 4 and 5 were given when a mean of 1-5, 6-10, 10-20 and > 20 inclusion (actively replicating organisms) positive cells was present.

The results for *C. psittaci* replication in the lungs and spleen, at 10 dpi, are shown in Table 10.

Table 10. Scores for chlamydia replication in the lungs and the spleen at 10 dpi.

Group	Chicken number	Lungs	Spleen	Avg score/bird
1	1	2	1	
	2	0	0	
	3	1	1	
	4	1	0	
	5	0	0	
	6	1	1	8
2a	1	2	2	
	2	2	2	
	3	1	1	
2b	1	2	2	
	2	1	1	
	3	1	1	18
3	1	3	3	
	2	3	2	
	3	3	3	
	4	2	3	
	5	2	3	
	6	3	2	32

5.3. Antibody responses

Enzyme-linked immunosorbent assays (ELISA's) were performed on the serum samples that first were pretreated with kaolin to remove background activity (Novak et al., 1993). Antibody titers were determined using standard protocols with rMOMP as antigen coated on the plates

- (Verminnen et al., 2006). Recombinant MOMP was produced in COS-7 cells, transiently transfected with pcDNA1::MOMP, as described previously (Vanrompay et al., 1998). Dilutions (1/1000) of, anti-chicken/turkey IgG, anti-chicken/turkey IgM and anti-chicken/turkey IgA (Bethyl Lab Inc, Montgomery, USA) were used. Anti-MOMP immunoglobulin titers were presented as the reciprocal of the highest serum dilution that gave an optical density (OD₄₀₅) above the cut-off value (mean OD off sero-negative SPF turkeys $\pm$ twice the S.D.). Also, a positive and negative control serum, obtained from previous vaccination experiments, was used. Starting dilution was 1/32.
- MOMP specific IgA, IgM, IgG serum antibody titers were determined by the recombinant MOMP-based ELISA (Verminnen et al., 2006) (Table 11). All SPF chickens tested sero-negative at the age of 6 weeks, at the beginning of our experiment.

Table 11. Serum IgM, IgG and IgA titers

Group	Chicken number	Challenge			Euthanasia		
		IgA	IgM	IgG	IgA	IgM	IgG
1	1	32	32	64	64	128	256
	2	0	32	64	32	64	256
	3	0	32	0	32	32	128
	4	32	32	64	64	64	256
	5	0	0	0	32	32	64
	6	0	32	0	32	64	128
2a	1	0	32	32	32	128	256
	2	0	0	0	32	64	32
	3	0	0	0	0	64	32
2b	1	32	64	32	64	64	128
	2	32	32	0	64	128	256
	3	0	32	0	32	128	64
3	1	0	0	0	32	128	128

2	0	0	0	0	128	64
3	0	0	0	32	256	64
4	0	0	0	0	128	64
5	0	0	0	0	128	128
6	0	0	0	32	256	128

Conclusion vaccination trial 2

Chickens immunized with a combination of B cell + CD4Th2 epitopes of MOMP together with a combination of CD8 + a CD4Th1 epitopes of MOMP (group 1) were better protected than non-immunized controls (group 3). Group 1 was also better protected than chickens immunized with either a combination of only B cell + CD4Th2 epitopes (group 2b) or a combination of only CD8 + CD4Th1 epitopes (group 2a).

Example 2 : *C. psittaci* recombinant MOMP vaccination experiment

Materials and methods

Chlamydia psittaci strain

C. psittaci strain 92/1293, isolated from a severe outbreak of respiratory disease in a commercial broiler turkey farm in the Netherlands, was used (Vanrompay et al., 1993). The strain was isolated from a pooled homogenate of the lungs, the cloacae and the spleens of diseased turkeys and was characterized as serovar D and genotype D (Geens et al., 2005). Bacteria were grown in Buffalo Green Monkey (BGM) cells as previously described (Vanrompay et al., 1992) and the titration was performed by the method of Spearman and Kaerber (Mayr et al., 1974).

Recombinant MOMP vaccine

Recombinant MOMP was produced in COS-7 cells transfected with the pcDNA1::MOMP plasmid, as previously described (Vanrompay et al., 1998). The plasmid contained the full-length *ompA* gene of *C. psittaci* strain 92/1293. After harvesting the recombinant MOMP (rMOMP), the protein concentration was determined using the bicinchoninic acid protein assay (Sigma).

Vaccination trial

Experiments were performed in negative pressure isolators (IM 1500, Montair Sevenum, the Netherlands). The experimental design was evaluated and approved by the Ethical Commission for Animal Experiments of Ghent University. To evaluate the *C. psittaci* rMOMP vaccine, 10 specific pathogen free (SPF) turkeys (CNEVA, Ploufragan, France) were divided into two groups. The vaccination scheme is presented in Table 12. The vaccinated group received 500 µg rMOMP per animal. The vaccine was administered as an aerosol using the CirrusTM nebulizer (2-5 µm aerosol particle size; Laméris, Aartselaar, Belgium). The control animals received no vaccine. Vaccination was performed on day 1 and at the age of 3 weeks. At the age of 5 weeks, all animals were aerogenically infected using a CirrusTM nebulizer (2-5 µm aerosol particle size; Laméris, Aartselaar, Belgium). The experimental infection dose in each isolator was 10⁶ TCID₅₀.

Table 12. Vaccination scheme

Group	n	Primo vaccination (day 1) and Dose	Booster vaccination (week 3)
1	5	rMOMP aerosol	500 µg/animal
2	5	Non-vaccinated control	/

Monitoring and sampling

Clinical signs were scored daily until necropsy. Clinical score 0 indicated no clinical signs; score 1: conjunctivitis; score 2: rhinitis; score 3: dyspnoea; score 4: conjunctivitis and rhinitis; score 5: conjunctivitis and dyspnoea; score 6: rhinitis and dyspnoea; score 7: conjunctivitis, rhinitis and dyspnoea.

Pharyngeal and cloacal excretion of *C. psittaci* was monitored on day 1 and on every other day starting at 5 days post infection (p.i.) until necropsy at 21 days p.i., using rayon-tipped, aluminium shafted swabs (Colpan; Fiers, Kuurne, Belgium) provided with *C. psittaci* transport medium (sucrose 74,6 g/l (Acros Organics, Geel, Belgium); KH₂PO₄ 5,1 g/l and K₂HPO₄ 1,2 g/l (Sigma); L-glutamic acid mono potassium salt 0,9 g/l (Invitrogen, Merelbeke, Belgium) and fetal calf serum 10% v/v (Greiner, Wommel, Belgium); gentamycin 50 µg/ml (Invitrogen);

vancomycin 100 µg/ml and streptomycin 100 µg/ml (Soenen, Merelbeke, Belgium); nystatin 25000 U/ml (Sigma) pH 7). Swabs were stored at -80°C until processed.

Blood samples for the detection of MOMP-specific serum antibody titres were collected prior to each vaccination, 7 days following the booster vaccination, immediately prior to the experimental infection and at 14 and 21 days p.i. Blood samples were stored overnight at room temperature, centrifuged (325×g, 10 min, 4 °C) and afterwards serum was collected and stored at -20 °C.

At the time of euthanasia, 21 days p.i., proliferative responses in peripheral blood lymphocytes were examined and characterized. All turkeys were examined for gross lesions. The score system is presented in Table 13. Cryostat tissue sections of the lungs, thoracic and abdominal airsacs, the pericardium, liver and spleen were examined for the presence of chlamydial antigen.

Table 13. Scores for *C. psittaci* macroscopic lesions in turkeys

Tissue	Score 1	Score 2	Score 3
Lungs	Slightly congested	Severely congested	Grey foci
Thoracic air sacs	Diffuse opacity	Focal fibrin deposits	Severe fibrinous airsacculitis
Abdominal air sacs	Diffuse opacity	Few fibrin deposits	Severe fibrinous airsacculitis
Pericardium	Serous pericarditis	Sero-fibrinous pericarditis	Fibrinous adhesive pericarditis
Spleen	Slightly enlarged	Moderately enlarged	Severely enlarged
Liver	Slightly enlarged	Moderately enlarged	Severely enlarged

Chlamydia replication in tissues and bacterial excretion

Cryostat tissue sections (5 µm) of the conchae, the conjunctivae, the trachea, the lungs, thoracic and abdominal airsacs, the pericardium, liver and spleen were examined for *C. psittaci* replication by the IMAGENTM immunofluorescence staining (IMAGENTM CHLAMYDIA, Oxoid, Drongen, Belgium), as previously described (Vanrompay et al., 1994). *C. psittaci* positive cells were enumerated in five randomly selected microscopic fields (400x, Nikon Eclipse TE2000-E, Japan)

and scored between 0 and 5: score 0 indicated no *C. psittaci* present; score 1 was given when a mean of 1-5 elementary bodies (infectious, non-metabolic morphological form) was present; score 2, 3, 4 and 5 were given when a mean of 1-5, 6-10, 10-20 and > 20 inclusion (actively replicating organisms) positive cells was present.

5 All swabs were examined for *C. psittaci* excretion by bacterial culture in Buffalo Green Monkey (BGM) cells (Vanrompay et al., 1992) and subsequent chlamydial identification using the IMAGENTM direct immunofluorescence assay (Vanrompay et al., 1992). The presence of *C. psittaci* was scored as for bacterial replication in different tissues.

10 *Antibody responses*

Enzyme-linked immunosorbent assays (ELISA's) were performed on the serum samples that first were pretreated with kaolin to remove background activity (Novak et al., 1993). Antibody titers were determined using standard protocols with rMOMP as antigen coated on the plates (Verminnen et al., 2006). Recombinant MOMP was produced in COS-7 cells, transiently
15 transfected with pcDNA1::MOMP, as described previously (Vanrompay et al., 1998). Dilutions of 1/2000 and 1/4000 of, respectively, biotinylated anti-chicken/turkey IgG (H + L) antibody and peroxidase-conjugated streptavidin were used. Anti-MOMP immunoglobulin titers were presented as the reciprocal of the highest serum dilution that gave an optical density (OD₄₀₅) above the cut-off value (mean OD off sero-negative SPF turkeys $\pm$ twice the S.D.). Also, a
20 positive and negative control serum, obtained from previous vaccination experiments, was used. Starting dilution was 1/32.

MOMP-specific isotypes in pharyngeal swabs were determined using cross-reactive anti-chicken IgG-, IgM- and IgA specific peroxidase-conjugated polyclonal antibodies (Bethyl Laboratories Inc.). Also, a positive and negative control serum, obtained from previous vaccination
25 experiments, was used. Starting dilution for the mucosal swabs was 1/32.

Lymphocyte proliferative responses and characterization of T cells

Peripheral blood leukocytes (PBL) were isolated from heparinized blood samples obtained by venepuncture (*V. ulnaris*) from each turkey, at 21 days p.i. Lymphocyte proliferative tests were
30 performed as previously described (Vanrompay et al., 1999). Briefly, non-adherent cells were grown in duplicate in 96-well tissue culture plates at 1×10^6 cells in 150 μ l of DMEM (Invitrogen)

supplemented with 20% heat-inactivated fetal calf serum (Greiner), 1% nonessential amino acids, 1% sodium pyruvate, 1% l-glutamine, 1% gentamycine and 0,1% β -mercaptoethanol (all Invitrogen). For antigen proliferation, 10 μ g of recombinant MOMP was added to individual wells. Negative and positive controls included cells stimulated with either plain medium or with 10 μ g concanavalin A (Con A), respectively. Cells were incubated at 39,5 °C in a humidified incubator with 5% CO₂. Con A or antigen induced proliferation was measured by incorporation of ³H-thymidine (1 μ Ci/well) during at last 16 h of culture, at days 2 (ConA) and 5, respectively. Cultures were harvested onto glass fiber filter strips with a cell harvester (Skatron, Liers, Norway). Filters were counted in a Beckman β -scintillation counter (Beckman, Gent, Belgium). The stimulation index was defined as the ratio of counts per minute (cpm) of stimulated cultures on medium-only cultures.

Proliferating cells were also characterized by flow cytometry with a cross reacting chicken anti-CD4 monoclonal antibody and a turkey anti-CD8 monoclonal antibody.

Statistical analysis

The Mann-Whitney test will be used for statistical analysis. Results will be considered significantly different at the level of $P < 0,05$.

5 Results

Clinical signs

Clinical signs in the rMOMP vaccinated group and the control group are presented in Table 14.

Table 14. Clinical signs at different days post infection (p.i.)

Days p.i.	rMOMP vaccin (n = 5)	Controls (n = 5)
4	Conjunctivitis (2)*	Lethargia, anorexia, Conjunctivitis (5)
7	Conjunctivitis (2), rhinitis (2)	Conjunctivitis (5), rhinitis (5)
9	Conjunctivitis (2), rhinitis (2), watery droppings (3)	Conjunctivitis (5) + rhinitis (5) + moderate dyspnee (3)
11	Conjunctivitis (2), rhinitis (3), Watery droppings (3)	Conjunctivitis (5) + rhinitis (5) + severe dyspnee (5), Watery droppings (3)
13	Conjunctivitis (2), moderate dyspnee (1)	Conjunctivitis (5) + rhinitis (5) + moderate dyspnee (3), severe dyspnee (2), Watery droppings (5)
15	Conjunctivitis (2), moderate dyspnee (2)	Conjunctivitis (5) + rhinitis (5) + moderate dyspnee (5), Watery droppings (4)
17	moderate dyspnee (2)	Conjunctivitis (5), rhinitis (3), moderate dyspnee (2), watery droppings (5),
19	/	Conjunctivitis (5) , rhinitis (3), moderate dyspnee (3), Watery droppings (4)
21	/	Conjunctivitis (5), rhinitis (3), Watery droppings (2), moderate dyspnee (3)

* The number of animals with clinical symptoms

Macroscopic findings

The macroscopic lesions in all animals were evaluated at necropsy (Table 15). Overall, the control animals showed severe congestion of the lungs with inflammatory sites and the air sacs showed a diffuse opacity with large fibrin deposits. Congestion of the conchae and conjunctivae could be observed in these animals and some of them also showed tracheal congestion. Moreover, a serous pericarditis was found in the control animals and also congestion of the liver and the spleen could be visualized.

The vaccinated animals, showed the most severe symptoms in the abdominal air sacs.

10 *Chlamydia replication and excretion*

The results for *C. psittaci* replication in the different tissues, at 21 days p.i., are shown in Table 16. The results of the pharyngeal and cloacal swabs that were analysed for *C. psittaci* excretion are shown in Table 17. All swabs that were taken from the one-day-old turkeys were negative.

15 *Antibody responses*

MOMP IgG (H + L), serum antibody titers were determined performed using our recombinant-MOMP based ELISA (Verminnen et al., 2006). The log₁₀ of the mean values of the antibody titers in function of the age of the turkeys is presented in Table 18.

Mucosal (pharyngeal) swabs taken at the day of the booster vaccination, at the day of the challenge infection and at euthanasia were investigated for the presence of IgG, IgM and IgA antibodies. The results are presented in Table 19.

Table 15. Mean scores per group $\pm$ S.D. (% positive turkeys) for *C. psittaci* macroscopic lesions on day 21 p.i.

Group	Conchae	Conjunctivae	Trachea	Lungs	Abdominal air sacs
rMOMP	1 $\pm$ 0 (100)	0 $\pm$ 0 (0)	0 $\pm$ 0 (0)	0.40 $\pm$ 0.89 (40)	0.60 $\pm$ 0.55 (60)
Controls	1.90 $\pm$ 0.55 (80)	0.50 $\pm$ 0.71 (60)	0.14 $\pm$ 0.38 (20)	1.90 $\pm$ 0.55 (80)	2.20 $\pm$ 1.10 (100)
Group	Thoracal air sacs	Pericardium	Liver	Spleen	Total score
rMOMP	0.20 $\pm$ 0.45 (20)	1 $\pm$ 0 (50)	0 $\pm$ 0 (0)	0.20 $\pm$ 0.45 (20)	3.4
Controls	1.90 $\pm$ 0.55 (100)	1.80 $\pm$ 0.84 (100)	0.60 $\pm$ 0.55 (80)	1.90 $\pm$ 0.55 (80)	12.84

Table 16. Mean score per group $\pm$ S.D. (% positive turkeys) for the presence of *C. psittaci*.

Group	Conchae	Conjunctivae	Trachea	Lungs	Abdominal air sacs
rMOMP	0.60 $\pm$ 0.55 (100)	1.80 $\pm$ 0.84 (20)	1.0 $\pm$ 0 (20)	0.60 $\pm$ 0.55 (40)	1.90 $\pm$ 0 (100)
Controls	2.20 $\pm$ 1.10 (100)	2.20 $\pm$ 1.10 (100)	2.20 $\pm$ 1.10 (100)	2.20 $\pm$ 0 (100)	3.00 $\pm$ 1.22 (100)
Group	Thoracal air sacs	Pericardium	Liver	Spleen	Total score
rMOMP	1.0 $\pm$ 0	1.00 $\pm$ 0	0.20 $\pm$ 0.45	1.00 $\pm$ 0.71	9.1

	(80)	(60)	(20)	(80)	
Controls	2.20 ± 0 (100)	2.20 ± 0 (100)	1.0 ± 0 (80)	1.0 ± 0 (80)	18.2

Table 17. Mean score per group ± S.D. (% positive turkeys) for pharyngeal and cloacal *C. psittaci* shedding.

Days PI	Pharyngeal excretion		Cloacal excretion	
	rMOMP	Control	rMOMP	Control
5	0.75 ± 0.50 (80)	1.60 ± 0.0 (100)	0.00 ± 0.00 (100)	0.75 ± 0.50 (20)
7	1.60 ± 0.89 (80)	1.80 ± 0.84 (100)	1.80 ± 0.80 (100)	1.60 ± 0.89 (40)
9	2.0 ± 1.00 (100)	2.20 ± 1.10 (80)	2.6 ± 0.9 (100)	3.0 ± 0 (80)
11	2.20 ± 1.10 (100)	3.00 ± 1.22 (100)	2.6 ± 0.9 (100)	2.20 ± 1.10 (100)
13	0.80 ± 1.3 (100)	3.00 ± 1.22 (100)	1.2 ± 1.1 (100)	3.00 ± 0.00 (100)
15	1.4 ± 0.90 (80)	3.00 ± 0.00 (100)	1.2 ± 1.1 (80)	3.00 ± 0.00 (100)
17	1.4 ± 0.90 (80)	2.20 ± 1.10 (100)	2.2 ± 1.10 (80)	3.00 ± 0.00 (100)
19	1.00 ± 0.71 (80)	1.80 ± 0.84 (80)	1.4 ± 0.9 (60)	3.00 ± 1.22 (80)
21	1.20 ± 1.10 (60)	2.20 ± 1.10 (100)	0.80 ± 1.3 (60)	2.20 ± 1.10 (100)

Table 18. Log₁₀ of mean serum antibody titers

Group	Preserum	BV ^a	One week PBV ^b	Challenge	2 weeks PC ^c	Euthanasia
rMOMP	0	1.34	2.61	2.60	2.10	2.03
Controls	0	0	0	0	1.86	2.08

^a BV, booster vaccination.^b PBV, post booster vaccination.5 ^c PC, post challenge**Table 19. Mean OD value per group for IgA, IgM and IgG in pharyngeal swabs**

Group	BV ^a	Challenge	Euthanasia
rMOMP			
IgA	0	0	0.100
IgM	0.124	0.111	0.152
IgG	0.110	0.107	0.122
Controls			
IgA	0	0	0.166
IgM	0	0	0.197
IgG	0	0	0.185

^a BV, booster vaccination.

10

Antigen-specific lymphocyte proliferation and characterization of T cells

The proliferative response of the peripheral blood lymphocytes after stimulation with recombinant MOMP of all vaccinated and control animals was determined on day 21 p.i. (Table 20).

15 The lymphocytes of the rMOMP vaccinated group showed a significant higher proliferative response compared to the control group.

Tabel 20. Proliferative response of the peripheral blood lymphocytes on day 21 p.i.

Group	Mean stimulation-index (S.I.) $\pm$ S.D.
rMOMP	4. $\pm$ 0.00
Control	1.30 $\pm$ 0.21

The proliferating cells were also characterized by flow cytometry with a cross-reacting chicken anti-CD4 monoclonal antibody and a turkey anti-CD8 monoclonal antibody. Results are shown in

Table 21.

Table 21. Lymphocyte CD4/CD8 characterization on day 21 p.i.

Group	Mean CD4/CD8 score $\pm$ S.D.
rMOMP	1,15 $\pm$ 0,31
Control	0,74 $\pm$ 0,29

CONCLUSION

Vaccination with the full-length recombinant MOMP was significantly less protective than the use of a combination of B and T cell epitopes of the MOMP (groups 4 and 6 of vaccination trial 1, and group 1 of vaccination trial 2 (vac.2) of the experiments in example 1) as:

- 1) **Clinical signs** in rMOMP vaccinated animals were significantly more severe than in animals of groups 4, 6 and 1 (vac.2). rMOMP vaccinated animals showed conjunctivitis, rhinitis, watery droppings and moderate dyspnea during the experiment. Animals of group 4 showed only conjunctivitis and rhinitis while the animals of group 6 and 1 (vac.2) stayed healthy. rMOMP vaccinated animals became healthy at 19 days p.i., while clinical signs in group 4 already disappeared at 8 days p.i.
- 2) **Macroscopic lesions** were observed in 60% of the rMOMP immunized animals and in 6 of 9 (67%) examined tissues. Lesions were most severe in the abdominal airsacs. For

group 4, gross pathology was only present in 2 of 7 (28%) animals and in 1 of 10 (10%) examined tissues, namely the lung. For group 6, gross pathology was only present in 1 of 8 (12.5%) animals and like for group 4 only in 1 of 10 (10%) examined tissues, namely the lung. Group 1 (vac.2) showed no macroscopic lesions.

5 3) **Bacterial excretion.** Pharyngeal and cloacal excretion in rMOMP vaccinated animals was higher than in groups 4 and 6.

4) **Replication of *C. psittaci* in internal organs.** (see Table 22)

10 Animals receiving rMOMP twice were less protected than the ones receiving the peptide combinations once (group 4) or twice (group 6). The maximum number of animals with a *C. psittaci* positive tissue was 50%, 57% and 100% for respectively group 6, 4 and the rMOMP-vaccinated group. The maximum score for *C. psittaci* replication in tissues was 0.38, 0.57 and 1.90 for respectively group 6, 4 and the rMOMP-vaccinated group. For vac 2, group 1 was best protected as demonstrated by the immunofluorescence staining on impression smears of the lungs and the spleen.

15 The highest protection (achieved in group 6) level was correlated with a significant higher mucosal IgA titer at the day of challenge and a higher lymphocyte proliferative response of especially spleen lymphocytes at 21 days post infection.

20 rMOMP vaccination gave no significant difference for the CD4/CD8 ratio in peripheral blood as compared to the controls, while the peptide combination (group 4 and 6) gave a significantly higher CD4/CD8 than the controls at any time point post infection. For group 1 (vac. 2), protection was correlated with a higher number of animals showing IgG serum antibodies at the day of challenge.

Table 22. Mean scores per group $\pm$ standard deviation (% positive animals) for the presence of *C. psittaci* in several organs on day 21 p.i.

Tissue	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	rMOMP (twice)
Conjunctivae	1.07 $\pm$ 0.19 (100)	0.50 $\pm$ 0.29 (86)	0.50 $\pm$ 0.41 (71)	0.29 $\pm$ 0.49 (29)	0.86 $\pm$ 0.24 (100)	0.13 $\pm$ 0.35 (13)	0.60 $\pm$ 0.55 (100)
Conchae	1.00 $\pm$ 0.29 (100)	0.29 $\pm$ 0.39 (43)	0.86 $\pm$ 0.99 (86)	0.50 $\pm$ 0.50 (57)	0.93 $\pm$ 0.19 (100)	0.06 $\pm$ 0.18 (13)	1.80 $\pm$ 0.84 (20)
Sinus	0.79 $\pm$ 0.49 (86)	0.57 $\pm$ 0.53 (57)	0.36 $\pm$ 0.38 (57)	0.36 $\pm$ 0.48 (43)	0.79 $\pm$ 0.27 (100)	0.25 $\pm$ 0.27 (50)	/
Trachea	1.14 $\pm$ 0.38 (100)	0.43 $\pm$ 0.35 (71)	0.50 $\pm$ 0.29 (86)	0.29 $\pm$ 0.49 (29)	1.14 $\pm$ 0.24 (100)	0.25 $\pm$ 0.38 (38)	1.00 $\pm$ 0 (20)
Lungs	1.36 $\pm$ 0.38 (100)	0.57 $\pm$ 0.35 (86)	0.50 $\pm$ 0.41 (71)	0.43 $\pm$ 0.73 (43)	1.57 $\pm$ 0.79 (100)	0.38 $\pm$ 0.44 (50)	0.60 $\pm$ 0.55 (40)
Thoracal air sacs	0.93 $\pm$ 0.35 (100)	0.14 $\pm$ 0.24 (29)	0.21 $\pm$ 0.27 (43)	0.29 $\pm$ 0.49 (29)	1.21 $\pm$ 0.64 (100)	0.13 $\pm$ 0.23 (25)	1.00 $\pm$ 0 (80)
Abdominal air sacs	1.14 $\pm$ 0.48 (100)	0.64 $\pm$ 1.11 (43)	0.64 $\pm$ 0.38 (86)	0.07 $\pm$ 0.19 (14)	1.21 $\pm$ 0.39 (100)	0.13 $\pm$ 0.35 (13)	1.90 $\pm$ 0 (100)
Pericardium	0.64 $\pm$ 0.24 (100)	0.36 $\pm$ 0.38 (57)	0.43 $\pm$ 0.19 (86)	0.14 $\pm$ 0.38 (14)	0.50 $\pm$ 0.50 (57)	0.00 $\pm$ 0.00 (0)	1.00 $\pm$ 0 (60)
Spleen	1.71 $\pm$ 0.95 (86)	0.14 $\pm$ 0.37 (14)	1.57 $\pm$ 1.17 (86)	0.57 $\pm$ 0.98 (29)	1.71 $\pm$ 0.76 (100)	0.38 $\pm$ 0.69 (38)	1.00 $\pm$ 0.71 (80)
Liver	0.29 $\pm$ 0.57 (29)	0.00 $\pm$ 0.00 (0)	0.14 $\pm$ 0.24 (29)	0.21 $\pm$ 0.39 (29)	0.36 $\pm$ 0.48 (43)	0.13 $\pm$ 0.35 (13)	0.20 $\pm$ 0.45 (20)

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Claims

1. A composition comprising one or more peptides each of said peptides comprising one or more epitopes selected from the list consisting of a B-cell epitope, a CD4+ Th2 cell epitope, a CD4+ Th1 cell epitope and a CTL epitope; wherein said composition comprises at least a B-cell epitope, a CD4+ Th2 cell epitope, a CD4+ Th1 cell epitope and a CTL epitope, and wherein the epitopes are located in the major outer membrane protein (MOMP) of *Chlamydia psittaci* and wherein the composition does not comprise the full-length *Chlamydia* MOMP protein.
2. The composition according to claim 1 wherein the B-cell epitope consists of an amino acid sequence selected from the group consisting of: GTASATT (SEQ ID NO 92), GTDFNN (SEQ ID NO 93) and NPTLLGKA (SEQ ID NO 94), or a variant thereof, and wherein the CD4+ Th2 cell epitope, the CD4+ Th1 cell epitope and the CTL epitope independently consist of an amino acid sequence selected from the group consisting of SEQ ID NO 20 and 101-117, or a variant thereof.
3. The composition according to claims 1 or 2, wherein
 - the B-cell epitope consists of an amino acid sequence selected from the group consisting of: GTASATT (SEQ ID NO 92), GTDFNN (SEQ ID NO 93) and NPTLLGKA (SEQ ID NO 94), or a variant thereof;
 - the peptide comprising a CD4+ Th2 cell epitope is selected from the group consisting of: SEQ ID NO 7, 8, 43, 76, 77, 85, and 91, or a variant thereof;
 - the peptide comprising a CTL (CD8+) epitope is selected from the group consisting of: SEQ ID NO 20, 26, 27, 42, 43, 61, 73, 75, and 80, or a variant thereof; and
 - the peptide comprising a CD4+ Th1 cell epitope is selected from the group consisting of: SEQ ID NO 1, 10-13, 18, 20, 25, 27, 29, 31-32, 35, 42, 46, 51, 53-56, 61, 64, 65-67, 69-73, 75, 79-81, and 87, or a variant thereof.
4. The composition according to claim 3, wherein the peptide comprising a B-cell epitope and/or a CD4+ Th2 cell epitope is selected from the group consisting of: SEQ ID NO 7, 8, 76, 77, 91 and 94-98, or a variant thereof, wherein the peptide comprising a CTL-cell epitope is selected from the group consisting of: SEQ ID NO 73, 75, 80 and 99, or a variant thereof, and

wherein the peptide comprising a CD4⁺ Th1 cell epitope is selected from the group consisting of SEQ ID NO 70, 71, 73, 75, 79, 80, 81 and 99, or a variant thereof.

5. The composition according to any one of claims 1 to 4, comprising or consisting of the following peptides: EPSLLIDGTMWEGASGDPCDPC (SEQ ID NO 97), TGTASATT (SEQ ID NO 95), KGTFDFNNQ (SEQ ID NO 96), AQPKLATAVLDLTTWNPTLLGKATTVDGTNTYSDFL (SEQ ID NO 98), and AATDTKSATLKYHEWQVGLALSRYRLNMLVPYIGVNWSRATFDADT (SEQ ID NO 99), or variants of thereof; or comprising or consisting of the following peptides: TWCDASIRAGYYGD (SEQ ID NO 20), EMLNVTSSPAQFVIH (SEQ ID NO 62), and KGTFDFNNQ (SEQ ID NO 96), or variants thereof.
6. The composition according to any one of claims 1 to 5, wherein part or all of the peptides are present as a mixture or are part of a polyepitope construct.
7. A composition comprising a nucleic acid sequence encoding the peptide as provided in any one of claims 1 to 5.
8. A composition according to any one of claims 1 to 7 further comprising an antigen delivery system.
9. A composition according to any one of claims 1 to 8 further comprising an adjuvant.
10. A composition according to any one of claims 1 to 9, for use as a medicament.
11. A composition according to any one of claims 1 to 9, for use in inducing an immunoprotective response in a subject against an infection with a species of the genus *Chlamydia*, in particular *Chlamydia psittaci*.
12. A composition according to any one of claims 1 to 9, for use as a vaccine against an infection with a species of the genus *Chlamydia*, in particular *Chlamydia psittaci*.
13. A vector comprising a nucleic acid sequence as provided in claim 7.
14. A cell comprising the vector according to claim 13.
15. An in vitro method to diagnose an infection with *Chlamydia psittaci* in a subject comprising:
 - contacting a sample from said subject with at least one peptide comprising a B-cell epitope as provided in claim 2 or 3, and
 - determining the presence of antibodies in said sample binding to said peptide(s).
16. A diagnostic kit comprising one or more peptides comprising a B-cell epitope as provided in claim 2 or 3.

Figure 1

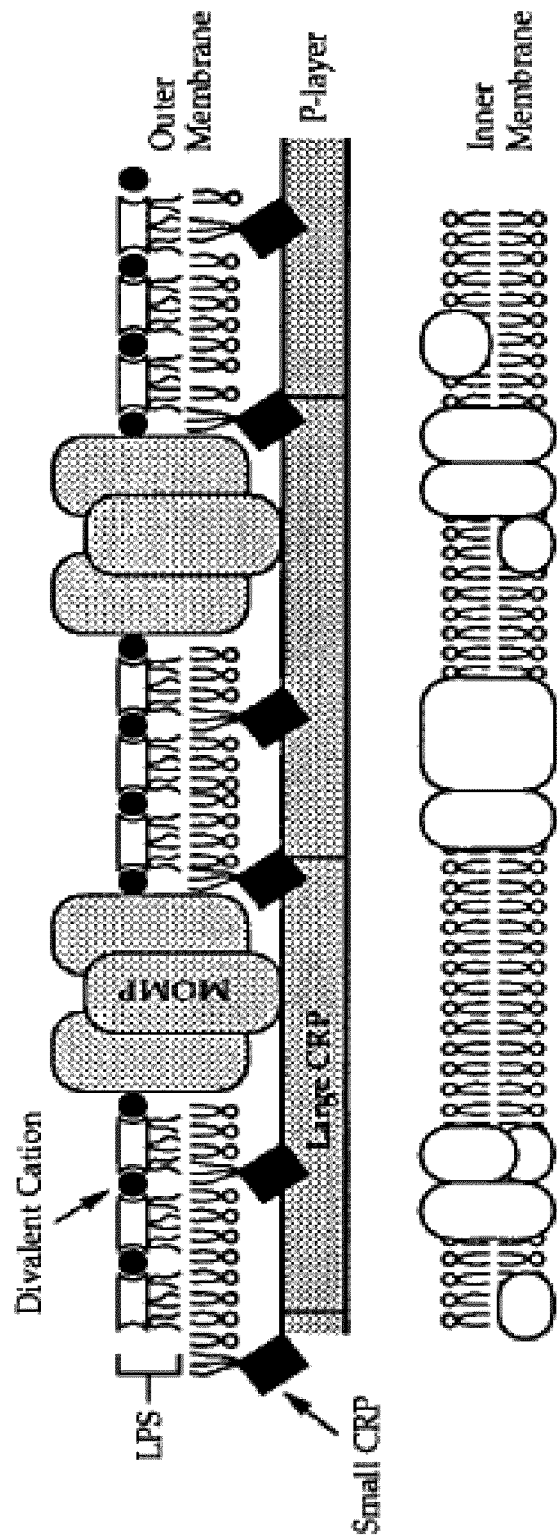


Figure 2

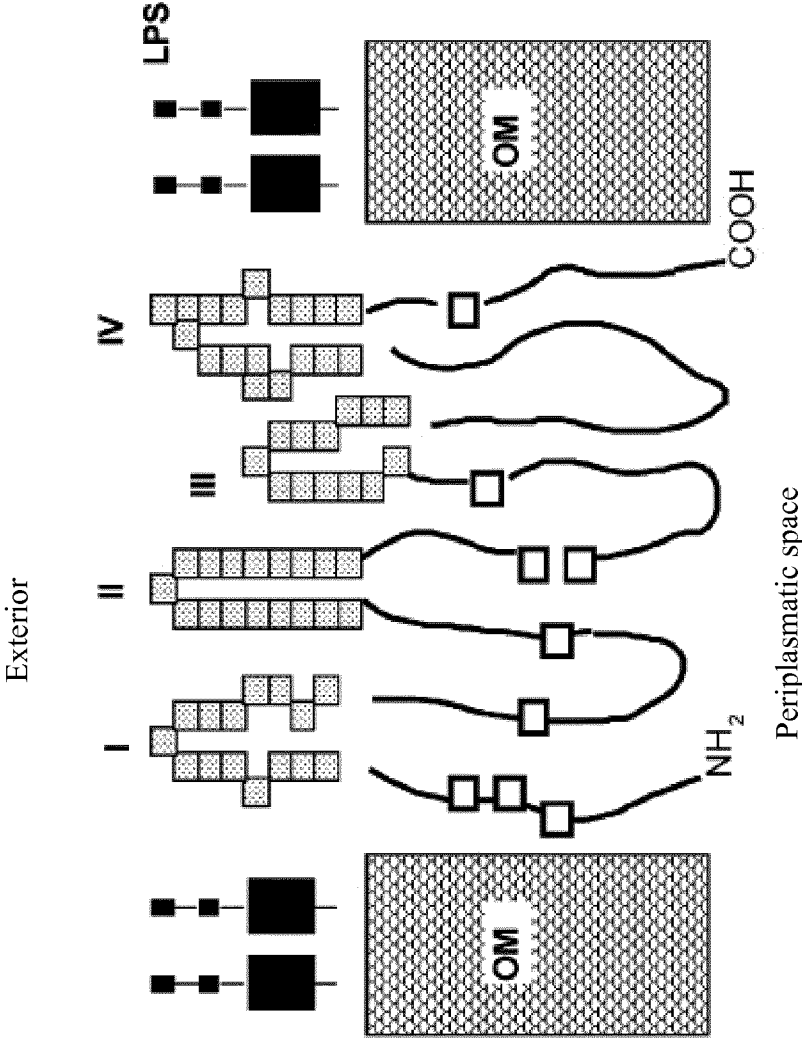


Figure 3

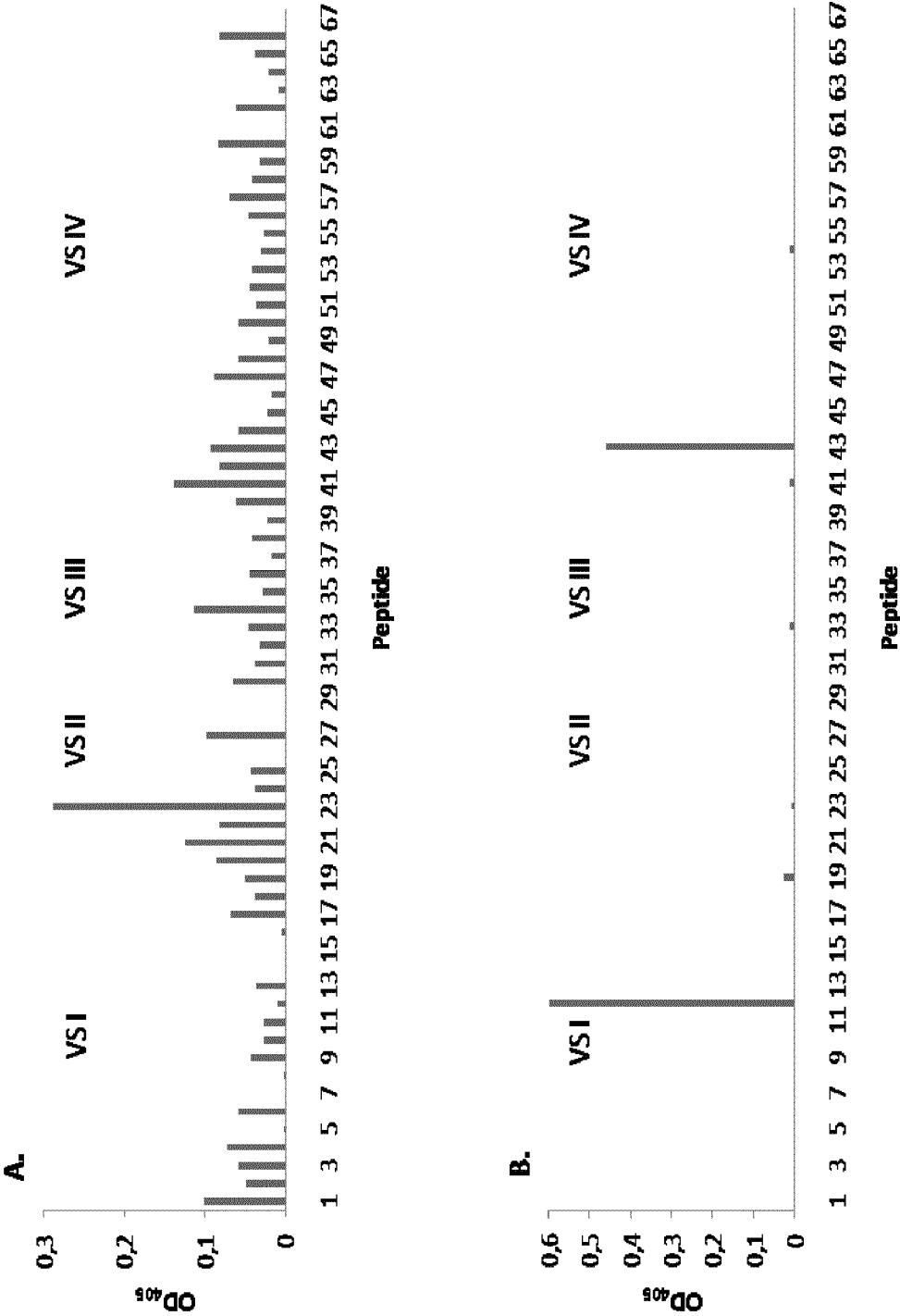


Figure 4

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Outer membrane protein A genes		1	75
OmpA_Ctra_NC000117_CT681_RC	(1)	-----	-----ATGAAAAA
OmpA_Cmur_AE002160_TC0052_RC	(1)	-----	-----ATGAAAAA
OmpA_Cppn_NC000922_CPn0695	(1)	-----	-----ATGAAAAA
OmpA_Cpfe1_NC007899_CF0958	(1)	-----	-----ATGAAAAA
OmpA_Cpcav_AE015925_CCA00047_RC	(1)	-----	-----ATGAAAAA
OmpA_Cpabo_NC004552_CAB048_RC	(1)	-----	-----ATGAAAAA
OmpA_84-2334_AJ310735	(1)	-----	-----ATGAAAAA
OmpA_6BC_M73035	(1)	AGAAGAGCAAATTAGATAGCGAGCACAAAAAGATACTAAGCATAATCTTTAGAGGTGAGTATGAAAAA	-----ATGAAAAA
OmpA_CP3_AF269265	(1)	-----	-----ATGAAAAA
OmpA_GD_AF269261	(1)	-----	-----ATGAAAAA
OmpA_NJ1_AF269266	(1)	-----	-----AAA
PCR Omp1_D_92/1293	(1)	-----	-----ATGAAAAA
OmpA_MN-VR122-Cal10_AF269262	(1)	-----	-----
OmpA_VS225_AF269259	(1)	-----	-----ATGAAAAA
OmpA_WSRTE30_AY762613	(1)	-----	-----ATGAAAAA
OmpA_M56_AF269268	(1)	-----	-----
OmpA_WC_AF269269	(1)	-----	-----ATGAAAAA
Consensus	(1)	-----	ATGAAAAA
		76	150
OmpA_Ctra_NC000117_CT681_RC	(10)	CTCTTGAAATCGGTATTAGTATTGCCGC---TTTGAGTTCTGCTTCTCCTCTGCAAGCTGCTGCTGTTGGGAAT	CTCTTGAAATCGGTATTAGTATTGCCGC---TTTGAGTTCTGCTTCTCCTCTGCAAGCTGCTGCTGTTGGGAAT
OmpA_Cmur_AE002160_TC0052_RC	(10)	CTCTTGAAATCGGTATTAGTATTGCCGC---TTTGAGTTCTGCTTCTCCTCTGCAAGCTGCTGCTGTTGGGAAT	CTCTTGAAATCGGTATTAGTATTGCCGC---TTTGAGTTCTGCTTCTCCTCTGCAAGCTGCTGCTGTTGGGAAT
OmpA_Cppn_NC000922_CPn0695	(10)	CTCTTAAAGTCGGCGTTATTATCCGCCGCAATTGCTGTTGCTTGGCTCCTTACAAGCCTTGCTGCTGTTAGGAAC	CTCTTAAAGTCGGCGTTATTATCCGCCGCAATTGCTGTTGCTTGGCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
OmpA_Cpfe1_NC007899_CF0958	(10)	CTCTTAAATCGGCATTATTATTGCCGC---TGCGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAT	CTCTTAAATCGGCATTATTATTGCCGC---TGCGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAT
OmpA_Cpcav_AE015925_CCA00047_RC	(10)	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAT	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAT
OmpA_Cpabo_NC004552_CAB048_RC	(10)	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
OmpA_84-2334_AJ310735	(10)	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
OmpA_6BC_M73035	(76)	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
OmpA_CP3_AF269265	(10)	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
OmpA_GD_AF269261	(10)	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
OmpA_NJ1_AF269266	(4)	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
PCR Omp1_D_92/1293	(10)	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
OmpA_MN-VR122-Cal10_AF269262	(1)	-----	-----CAAGCCTTGCTGTTAGGAAC
OmpA_VS225_AF269259	(1)	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
OmpA_WSRTE30_AY762613	(10)	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC	CTCTTGAAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
OmpA_M56_AF269268	(10)	-----AAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC	-----AAATCGGCATTATTATTGCCGC---TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
OmpA_WC_AF269269	(1)	CTCTTGAAATCGGCATTATTATTGCCGC	TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC
Consensus	(76)	CTCTTGAAATCGGCATTATTATTGCCGC	TACGGGTTCCGCTCTCTCCTTACAAGCCTTGCTGCTGTTAGGAAC

Figure 4 – continued (1) 5/23

OmpA_Ctra_NC000117_CT681_RC	(82)	151	225
OmpA_Cmur_AE002160_TC0052_RC	(82)		
OmpA_Cppn_NC000922_CPn0695	(85)		
OmpA_CpfeI_NC007899_CF0958	(82)		
OmpA_Cpcav_AE015925_CCA00047_RC	(82)		
OmpA_Cpabo_NC004552_CAB048_RC	(82)		
OmpA_84-2334_AJ310735	(82)		
OmpA_6BC_M73035	(148)		
OmpA_CP3_AF269265	(82)		
OmpA_GD_AF269261	(82)		
OmpA_NJ1_AF269266	(76)		
PCR Omp1_D_92/1293	(82)		
OmpA_MN-VR122-Cal10_AF269262	(22)		
OmpA_VS225_AF269259	(73)		
OmpA_WSRTE30_AY762613	(82)		
OmpA_M56_AF269268	(82)		
OmpA_WC_AF269269	(67)		
OmpA_Consensus	(151)		
OmpA_Ctra_NC000117_CT681_RC	(157)	226	300
OmpA_Cmur_AE002160_TC0052_RC	(157)		
OmpA_Cppn_NC000922_CPn0695	(160)		
OmpA_CpfeI_NC007899_CF0958	(157)		
OmpA_Cpcav_AE015925_CCA00047_RC	(157)		
OmpA_Cpabo_NC004552_CAB048_RC	(157)		
OmpA_84-2334_AJ310735	(157)		
OmpA_6BC_M73035	(223)		
OmpA_CP3_AF269265	(157)		
OmpA_GD_AF269261	(157)		
OmpA_NJ1_AF269266	(151)		
PCR Omp1_D_92/1293	(157)		
OmpA_MN-VR122-Cal10_AF269262	(97)		
OmpA_VS225_AF269259	(148)		
OmpA_WSRTE30_AY762613	(157)		
OmpA_M56_AF269268	(157)		
OmpA_WC_AF269269	(142)		
OmpA_Consensus	(226)		

Figure 4 – continued (2)

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OmpA_Ctra_NC000117_CT681_RC	(232)	301	GTGAATAAAGAAATTCAG---ATGGGTGCCAAGCCTACAACGTATACAGGCAATAGTGCAGCTCCATCCACTCTTT	375
OmpA_Cmur_AE002160_TC0052_RC	(232)		GTGAACAAACAGTTCGAA---ATGGGAGCAGCTCCTACAGGAGATGCAGACCTTACTACAGCACCT---ACTCCTT	
OmpA_Cppn_NC000922_CPn0695	(235)		GCACCTAAACCATTTTCT---ATGGGAGCCAGCCTACTGG-----ATCCGCTGCTGCAAACTATATACTAGTCC	
OmpA_CpfeI_NC007899_CF0958	(232)		GTTAATAAAACCATCAGCGGAATGGCTGGGCTCCAACAGAGCTTCTGGAACCTGCAAGCAACACACACTGTGCGT	
OmpA_Cpcav_AE015925_CCA00047_RC	(232)		GTTAATAAAACCTATCAGC---ATGGGACAGCTCCAACTGG---TAATGCAGCTGCTGACTTTAAACCCGT---T	
OmpA_Cpabo_NC004552_CAB048_RC	(232)		GTGAATAAAACCTATCACCGCATGGTGCAGTTCCTACAGG-----AACCGCAGCAGCTAATACAAAACCTCCT	
OmpA_84-2334_AJ310735	(232)		GTGAATAAAACCTTTACCGGCATGGTGCAGTTCCTACAGG-----AACTCAGCAGCTGATTTTAAAACTCCT	
OmpA_6BC_M73035	(298)		GTGAATAAAACCTTTAGCGGCATGGTGCAGTTCCTACAGGCTCCTACGCAAGCTACAGGTAACGCAAGTAATACTAATCAGCCA	
OmpA_CP3_AF269265	(232)		GTGAATAAAACCTTTAGCGGCATGGTGCAGTTCCTACAGGCTACAGGCTACAGGTAACGCAAGTAATACTAATCAGCCA	
OmpA_GD_AF269261	(232)		GTGAATAAAACCTTTACCGGCATGGTGCAGGTAACCCACAGG-----ATCCTCTCCAAATGACTTTTAAAAATGCT	
OmpA_NJ1_AF269266	(226)		GTGAATAAAACCTTTAGCGGCATGGTAAATCACCCTACAGAGGCTACAGGTAACGCAAGTAATACTACTACTGCT	
PCR_Omp1_D_92/1293	(232)		GTGAATAAAACCTTTAGCGGCATGGTAAATCACCCTACAGAGGCTACAGGTAACGCAAGTAATACTACTACTGCT	
OmpA_MN-VR122-Cal10_AF269262	(172)		GTGAATAAAACCTTTAGCGGCATGGTGCAGTTCCTACAGGCTACAGGTAACGCAAGTAATACTAATCAGCCA	
OmpA_VS225_AF269259	(223)		GTGAATAAAACCTTTAGCGGCATGGTGCAGTTCCTACAGG-----AAGCGCAGCAGCGGATACAAAACCTCCT	
OmpA_WSRTE30_AY762613	(232)		GTGAATAAAACCTTTAGCGGCATGGTGCAGTTCCTACAGGCTCCTACGCAAGCTACAGGTAACGCAAGTAATACTAATCAGCCA	
OmpA_M56_AF269268	(232)		GTGAATAAAACCTTTTACTGGAATGGCAGCAACTCCTACAGAGGCTTTCAGGTAACGCAACCAATACTAGTACGCC	
OmpA_WC_AF269269	(217)		GTGAATAAAACCTTTTAGCGGCATGGTGCAGTTCCTACGGAGTCTTTCAGGAACTGTTTCTCGGCTAAACAGCT	
Consensus	(301)		GTGAATAAAACCTTTTAGCGGCATGGTGCAGTTCCTACAG GCT CAGG AC GCAA T ATACTAATACTCCT	450
OmpA_Ctra_NC000117_CT681_RC	(304)	376	ACAGC--A-----AGAGAGAAATCTGCTTACGGCCGACATATGCAGGATGCTGAGATGTTTACAAAATGCCGCTTGC	
OmpA_Cmur_AE002160_TC0052_RC	(301)		GCATC--A-----AGAGAGAAATCCCGCTTATGGCAAGCATATGCAAGATGCAAAAATGTTCACTAATGCTGCGGTAC	
OmpA_Cppn_NC000922_CPn0695	(301)		GTAG--AT---AGACCTAACCCGGCTTACATAAGCATTTTACACGATGCAGAGTGGTTCACTAATGCAGGCTTC	
OmpA_CpfeI_NC007899_CF0958	(307)		GCG--AC---AGATCAAAATTTGCTTACGGCAAAACATCTCAAGATGCCAAATGGTGCAACCAATGCTGCTTAC	
OmpA_Cpcav_AE015925_CCA00047_RC	(298)		GCAG--AC---AGGAATAACATAGCCTACGGCAAAACATATGCAAGATGCAAAATGGTCCAAAACGCGGCTTTC	
OmpA_Cpabo_NC004552_CAB048_RC	(301)		ACGG--AT---AGACCCAAATCGCTTACGGCAAAACATTTACAAAGACGCCGAATGGTTTACCAATGCAGCTTTC	
OmpA_84-2334_AJ310735	(301)		ACAG--AT---AGAGCGAAATCGCTTATGSCAAGCACITTCGCAAGCAGCCGAATGGTTTACCAATGCCGCTTTC	
OmpA_6BC_M73035	(373)		GAAGCAAAATGGCAGACCCGATCGCTTACGGAAGGCATATGCAAGATGCAGAGTGGTTTCAAAATGCAGCTTC	
OmpA_CP3_AF269265	(307)		GAAGCAAAATGGCAGACCCGAAATCGCTTACGGAAGGCATATGCAAGATGCAGAGTGGTTTCAAAATGCAGCTTC	
OmpA_GD_AF269261	(301)		GAAG--AT---AGACCCAAATCGCTTATGSCAAGCACITTCGCAAGCAGCCGAATGGTTTACCAATGCAGCTTTC	
OmpA_NJ1_AF269266	(301)		GTAG--AT---AGAACCAATTTAGCTTATGGCAAAACATTTGCAAGATGCCAAATGGTTTACCAATGCAGCTTC	
PCR_Omp1_D_92/1293	(307)		GTAG--AT---AGAACCAATTTAGCTTATGGCAAAACATTTGCAAGATGCCAAATGGTTTACCAATGCAGCTTTC	
OmpA_MN-VR122-Cal10_AF269262	(247)		GAAGCAAAATGGCAGACCCGAAATCGCTTACGGAAGGCATATGCAAGATGCAGAGTGGTTTCAAAATGCAGCTTC	
OmpA_VS225_AF269259	(292)		ACAG--AT---AGACCCAAATCGCTTATGSCAAGCACITTCGCAAGCAGCCGAATGGTTTCAAAATGCAGCTTC	
OmpA_WSRTE30_AY762613	(307)		GAAGCAAAATGGCAGACCCGAAATCGCTTACGGAAGGCATATGCAAGATGCAGAGTGGTTTCAAAATGCAGCTTC	
OmpA_M56_AF269268	(307)		GAAGCAAAATGGCAGACCCGAAATCGCTTACGGAAGGCATATGCAAGATGCAGAGTGGTTTCAAAATGCAGCTTTC	
OmpA_WC_AF269269	(292)		GTAG--AT---AGAGTCAATCTTGCCTATGGGAAACATTTACAAAGATGCCGAGTGGTTTACAAATCTCTGCTTTC	
Consensus	(376)		G AG AT AGA C AACATCGCTTACGGCAAAACAT TGCAAGATGC GAGTGGTTTACAAATGCAGCTTTC	

Figure 4 – continued (3)

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OmpA_Ctra_NC000117_CT681_RC	(373)	ATGGCATTGAATATTTGGGATCGTTTTGATGTATTTCTGTACATTAGGAGCCACCAGTGGATATCTTAAAGGAAAC	525
OmpA_Cmur_AE002160_TC0052_RC	(370)	ATGGCTTTAAACATTTGGGACCGTTTCGATCTATTTTGTACATTTGGGAGCAACTAGCGGATATCTTAAAGGTAAT	
OmpA_Cppn_NC000922_CPn0695	(370)	ATTGCCCTTAAACATTTGGGATCGCTTTGATGTTTCTGTACTTTAGGAGCTTCTAATGGTTACATTAGAGGAAAC	
OmpA_CpfeI_NC007899_CF0958	(376)	TTAGCATTAAATATTTGGGATCGTTTTGATGTTTTCTGCACGCTAGGAGCGTTAATGGTTACTTCAAAGCAAAT	
OmpA_Cpcav_AE015925_CCA00047_RC	(367)	TTAGCATTAAACATTTGGGATCGTTTTGATGTTTCTGTGCACATTAGGGGCACTTAACGGCTATCTCAAAGCAAAT	
OmpA_Cpabo_NC004552_CAB048_RC	(370)	CTCGCATTGAATATCTGGGATCGCTTTGATATTTTCTGCACATTAGGCGCTTCTAATGGGTACTTCAAAGGTAGT	
OmpA_84-2334_AJ310735	(370)	CTCGCATTAAATATTTGGGATCGCTTTGACATTTTCTGCACATTAGGCGCTTCTAATGGCTACTTCAAAGGTAGT	
OmpA_6BC_M73035	(448)	CTAGCCTTAAACATTTGGGATCGCTTCGACATTTTCTGCACATTAGGGGCACTCAATGGATACTTCAAAGCAAAT	
OmpA_CP3_AF269265	(382)	CTAGCCTTAAACATTTGGGATCGCTTCGACATTTTCTGCACATTAGGGGCACTCAATGGATACTTCAAATCAAAT	
OmpA_GD_AF269261	(370)	TTAGCGTTAAATATCTGGGATCGTTTTGATATTTTCTGCACATTAGGCGCTTCTAATGGGTACTTCAAAGGTAGT	
OmpA_NJ1_AF269266	(370)	CTCGCATTAAATATCTGGGATCGCTTTGATATTTTCTGCACATTAGGGGCTTCTAATGGGTACTTCAAAGGTAGT	
PCR_Omp1_D_92/1293	(376)	CTCGCATTAAATATCTGGGATCGCTTTGATATTTTCTGCACATTAGGGGCTTCTAATGGGTACTTCAAAGGTAGT	
OmpA_MN-VR122-Cal10_AF269262	(322)	CTAGCCTTAAACATTTGGGATCGCTTCGACATTTTCTGCACATTAGGGGCACTCAATGGATACTTCAAATCAAAT	
OmpA_VS225_AF269259	(361)	CTCGCATTAAATATCTGGGATCGCTTTGATATTTTCTGCACATTAGGTGCTTCCAAATGGGTACTTCAAAGGTAGT	
OmpA_WSRTE30_AY762613	(382)	CTAGCCTTAAACATTTGGGATCGCTTCGACATTTTCTGCACATTAGGGGCACTCAATGGATACTTCAAAGCAAAT	
OmpA_M56_AF269268	(382)	CTAGCCTTAAACATTTGGGATCGCTTCGATATTTTCTGCACATTAGGATGCTTCTAATGGGTACTTCAAAGGAAAT	
OmpA_WC_AF269269	(361)	CTAGCATTAAACATTTGGGATCGCTTTGATATTTTCTGCACATTAGGTGCTTCTAATGGGTACTTCAAAGGAAAT	
OmpA_WC_AF269269	(451)	CTAGCATTAAACATTTGGGATCGCTTTGATATTTTCTGCACATTAGG GC TCTAATGG TACTTCAAAGCAAAT	600
OmpA_Ctra_NC000117_CT681_RC	(448)	TCTGCTTCTTCAATTTAGTTGGATTGTTTGGAGATAATGAAAAATCAAAAAAC--G-----GTCAAAAGCGGAGTCT	
OmpA_Cmur_AE002160_TC0052_RC	(445)	TCTGCCGCTTTAACTTAGTTGGTCTGTTTGGAGAGAGATGAACTGCA-----GTTGCAGCTGACGAC	
OmpA_Cppn_NC000922_CPn0695	(445)	TCTACAGCGTTCAATCTCGTTGGTTTATTTCGGAGTTAAAGGTACTACTGTAAA-----TGCAAATGAA	
OmpA_CpfeI_NC007899_CF0958	(451)	TCTGATGCATTAAACCTTGTGCGATTGATTGGTCTTGCAGGAACCTGATTT-----CGCCAAATCAG	
OmpA_Cpcav_AE015925_CCA00047_RC	(442)	GCTGCAGCTTCAATCTAGTCGGCTTACTTGGGGTAAACAGGAACAGATCT-----TCAAGGCCAA	
OmpA_Cpabo_NC004552_CAB048_RC	(445)	TCTGCCGCAATCAACCTCGTTGGTTTGAATGGTGTAAAGGATCCTCCATAG--C-----AGCTGATCAG	
OmpA_84-2334_AJ310735	(445)	TCCGGCGCAATCAATCTCGTCGGTTTGAATGGTGTAAAGGAAACACCTTAA--C-----AAATGACCGA	
OmpA_6BC_M73035	(523)	TCCGGTGCATTCAACCTTGGTTGGGTTAATAGGTTTTCAGCTGCAAGCTCAATCTCTACCGATCTTCCAAATGCAA	
OmpA_CP3_AF269265	(457)	TCCGGTGCATTCAACCTTGGTTGGGTTAATAGGTTTTCAGCTGCAAGCTCAATCTCTACCGATCTTCCAAATGCAA	
OmpA_GD_AF269261	(445)	TCTGCCGCAATCAATCTCGTTGGTTTGAATGGTGTAAAGGAAACCTCCCTAA--C-----AAATGACCAA	
OmpA_NJ1_AF269266	(445)	TCTGCCGCAATCAACCTCGTTGGTTTGAATGGTGTAAAGGAAACCTGATTT-----CAATATATCAA	
PCR_Omp1_D_92/1293	(451)	TCTGCCGCAATCAACCTCGTTGGTTTGAATGGTGTAAAGGAAACCTGATTT-----CAATATATCAA	
OmpA_MN-VR122-Cal10_AF269262	(397)	TCCGGTGCATTCAACCTTGGTTGGGTTAATAGGTTTTCAGCTACCACTCACTCTACCGAGCTTCCAAATGCAA	
OmpA_VS225_AF269259	(436)	TCTGCTGCATTCAACCTCGTTGGTTTGAATGGTGTAAAGGAAACCTCCGTAG--C-----AGCTGATCAA	
OmpA_WSRTE30_AY762613	(457)	TCCGGTGCATTCAACCTTGGTTGGGTTAATAGGTTTTCAGCTACCACTCACTCTACCGAGCTTCCAAATGCAA	
OmpA_M56_AF269268	(457)	TCTGCCGCAATCAACCTTGGTTGGGTTAATAGGTTTTCAGCTTCAAGCGGATAGTACCGATCTTCCAAAGCAA	
OmpA_WC_AF269269	(436)	TCCGCTGCTTCAATCTTGTGGCTTGTTCGGAATGTTCGAAATAGCGAAA---GTAATGCTCTTAAATGACCAA	
OmpA_WC_AF269269	(526)	TCTGC GCATTCAACCT GTTGG TTGATTGG TTAAAGGAAC CTTAA C AA CAA	

Figure 4 – continued (4)

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OmpA_Ctra_NC000117_CT681_RC	(517)	601	GTACCAAAATATGAGCTTTGATCAATCTGTTGTTGAGTTGATACAGATATCTACTTTTTCGCTGGAGCGTCGGCGCT	675
OmpA_Cmur_AE002160_TC0052_RC	(508)		ATACCTAAACGTCAGCTTGTCTCAAGCTGTTGTCGAACCTCTACACAGACACACAGCTTTCGCTTGGAGCGTCGGTGCT	
OmpA_Cppn_NC000922_CPn0695	(508)		CTACCAAAACGTTTCTTTAAAGTAACGGAGTTGTTGAACCTTTACACAGACACACCTTCTCTCTTGGAGCGTAGCGCT	
OmpA_CpfeI_NC007899_CF0958	(511)		CGTCCAAAACGTTGAAATTTCTCAAGGCAATTTGTAAGACTATACACAGATACCGCATTTCTTCTTGGAGCGTTGGTGCT	
OmpA_Cpcav_AE015925_CCA00047_RC	(502)		TATCCAAAACGTAGCCATCTCTCAAGGCCCTTGTAGAGCTTTATACTGACACACACCTTCTCTTGGAGCGTTGGTGCG	
OmpA_Cpabo_NC004552_CAB048_RC	(508)		CTTCCCAATGTAGGCATCATCCAAAGGAATCGTTGAATTTTATACAGATACACATCTCTTGGAGTGTAGTGCA	
OmpA_84-2334_AJ310735	(508)		CTTCCCAACGTAGGCATCATCCAAAGGCGTTGTGAGTTTATACAGATACAAATCTCTTGGAGCGTAGTGCA	
OmpA_6BC_M73035	(598)		CTTCCTAACGTAGGCATTTACCAAGGTTGTGGAATTTTATACAGACACATCATCTCTTGGAGCGTAGTGCA	
OmpA_CP3_AF269265	(532)		CTTCCTAACGTAGGCATTTACCAAGGTTGTGGAATTTTATACAGACACATCTCTTGGAGCGTAGTGCA	
OmpA_GD_AF269261	(508)		CTTCCCAACGTAGGCATCATCCAAAGGCGTTGTGAGTTTATACAGATACAACTCTCTTGGAGCGTAGTGCA	
OmpA_NJ1_AF269266	(505)		CTTCCCAACGTAGGCATCATCCAAAGGCGTTGTGAGTTTATACAGACACAACTCTCTTGGAGCGTAGTGCA	
PCR Omp1_D_92/1293	(511)		CTTCCCAACGTAGGCATCATCCAAAGGCGTTGTGAGTTTATACAGACACAACTCTCTTGGAGCGTAGTGCA	
OmpA_MN-VR122-Cal10_AF269262	(472)		CTTCCCTAACGTAGGCATTTACCAAGGTTGTGGAATTTTATACAGACACATCTCTTGGAGCGTAGTGCA	
OmpA_VS225_AF269259	(499)		CTTCCCAACGTAGGCATCATCCAAAGGTTGTGAGTTTATACAGATACAACTCTCTTGGAGCGTAGTGCA	
OmpA_WSRTE30_AY762613	(532)		CTTCCTAACGTAGGCATTTACCAAGGTTGTGGAATTTTATACAGACACATCTCTTGGAGCGTAGTGCC	
OmpA_M56_AF269268	(508)		CTTCCCAACGTAGGCATCATCCAAAGGTTGTGAGTTTATACAGATACAACTCTCTTGGAGCGTAGTGCA	
OmpA_WC_AF269269	(601)		CTTCC AACGTAGGCAT ACITCAAGG GTTGTGAGTTTATACAGACACAACTCTCTTGGAGCGTAGTGCA	750
OmpA_Ctra_NC000117_CT681_RC	(592)		CGCGCAGCTTTGTGGGAATGTGATGTGCAACTTTAGGAGCTTCATTCCAAATATGCTCAATCTAAACCTAAAGTA	
OmpA_Cmur_AE002160_TC0052_RC	(583)		AGAGCAGCTTTATGGGAGTGGGATGTGCAACTTTTAGGAGCTTCCTTCCAAATATGCTCAATCTAAAGCCAAAAGTA	
OmpA_Cppn_NC000922_CPn0695	(583)		CGTGGAGCCTTATGGGAATCGCGTTGTGCAACTTTGGAGCTGAAATCCAAATATGACACAGTCCAAACCTAAAGTT	
OmpA_CpfeI_NC007899_CF0958	(586)		CGCGGAGCTTTGTGGGAATGTGTTGTGCAACTTTGGGAGCTGAAATCCAAATATGCTCAATCCCAATCCCTAAATTT	
OmpA_Cpcav_AE015925_CCA00047_RC	(577)		CGTGGAGCTTTATGGGAATGTGTTGCGCACTTTAGGAGCAGAGTTCCAAATATGCGCAGTCTAATCCCTAAGATC	
OmpA_Cpabo_NC004552_CAB048_RC	(583)		CGCGGAGCTTTATGGGAGTGTGTTGTGCGACTTTAGGAGCAGAGTTCCAAATAGCTCAGTCAATCCCTAAATTT	
OmpA_84-2334_AJ310735	(583)		CGCGGAGCTTTATGGGAGTGTGCGTGTGCAACTTTAGGGGAGAAATCCAAATACGCTCAATCTAATCCCTAAATTT	
OmpA_6BC_M73035	(673)		CGTGGAGCTTTATGGGAATGTGTTGTGCAACTTTAGGAGCTGAGTTCCAAATACGCTCAATCTAATCCCTAAGATT	
OmpA_CP3_AF269265	(607)		CGTGGAGCTTTATGGGAATGTGTTGTGCAACTTTAGGAGCTGAGTTCCAAATACGCTCAATCTAATCCCTAAGATT	
OmpA_GD_AF269261	(583)		CGTGGAGCTTTATGGGAATGTGTTGTGCAACTTTAGGAGCTGAGTTCCAAATACGCTCAATCTAATCCCTAAGATT	
OmpA_NJ1_AF269266	(580)		CGTGGAGCTTTATGGGAATGTGTTGTGCGACTTTAGGAGCAGAGTTCCAAATACGCTCAATCTAATCCCTAAGATT	
PCR Omp1_D_92/1293	(586)		CGTGGAGCTTTATGGGAATGTGTTGTGCGACTTTAGGAGCAGAGTTCCAAATACGCTCAATCTAATCCCTAAGATT	
OmpA_MN-VR122-Cal10_AF269262	(547)		CGTGGAGCTTTATGGGAATGTGTTGTGCAACTTTAGGAGCTGAGTTCCAAATACGCTCAATCTAATCCCTAAGATT	
OmpA_VS225_AF269259	(574)		CGTGGTGTCTTATGGGAATGTGTTGTGCAACTTTAGGAGCTGAAATCCAGTATGCTCAATCTAATCCCTAAGATT	
OmpA_WSRTE30_AY762613	(607)		CGTGGAGCTTTATGGGAATGTGTTGTGCAACTTTAGGAGCTGAGTTCCAAATACGCTCAATCTAATCCCTAAGATT	
OmpA_M56_AF269268	(607)		CGTGGAGCTTTATGGGAATGTGTTGTGCGCACTTTAGGAGCTGAGTTCCAAATACGCTCAATCTAATCCCTAAGATT	
OmpA_WC_AF269269	(583)		CGCGGAGCTTTATGGGAGTGTGCTGCGCACTTTAGGAGCAGAAATCCAAATACGCAACATCTAATCCCTAAGATT	
Consensus	(676)		CGTGGAGCTTTATGGGAATGTGTTGTGCAACTTTAGGAGCTGAGTTCCAAATACGCTCAATCTAATCCCTAAGATT	

Figure 4 – continued (5)

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	OmpA_Ctra_NC000117_CT681_RC	(667)	GAAGAAATAAACGTTCTCTGTCAATCGACGACAGATTTCCTACTATTATAAACCTTAAAGGGTATGTAGGTAAGGAGT---	823
	OmpA_Cmur_AE002160_TC0052_RC	(658)	GAGGAATTAACCGTTCTCTGTAAATGGGCAGAAATTCACCTATTATAACAGCCTAAAGGATACGTTTGACAAGAGT--	
	OmpA_Cppn_NC000922_CPn0695	(658)	GAGAACTTAATGTGATCTGTAACTGATTCGCAATTCCTGTATAAACAAACCACAGGGCTATAAAGCGTGTGCTT--	
	OmpA_Cpfel_NC007899_CF0958	(661)	GAAATGCTCAATGTAACTTCTAGCCGACGACAATTCATCATACACAGCCTAGAGGATACATAAAGGACTTGCAGCA	
	OmpA_Cpcav_AE015925_CCA00047_RC	(652)	GAAATGCTTAATGTAAATTTCTAGCCAAACACAATTTGTGATTTCATPAAGCCTAGAGGATATATAAAGGACAGCGGCC	
	OmpA_Cpabo_NC004552_CAB048_RC	(658)	GAAATGTTGAATGTAGTCTCCAGCCGACGACAATTTGTGTTTCACAAGCCTTAGAGGATACAAGGGAACAGCAT--	
	OmpA_84-2334_AJ310735	(658)	GAAATGTTGAATGTAACTTTAGCCGACGACAATTTGTGTTTCACAAAACCTTAGAGGCTATAAAGGGGCAACAGCG	
	OmpA_6BC_M73035	(748)	GAAATGCTCAACGTCACCTTCAAGCCGACGACAATTTGTGATTTCACAAAACCAAGAGGCTATAAAGGAGCTAGTCTCG	
	OmpA_CP3_AF269265	(682)	GAAATFACTCAACGTCACTTCAAGCCGACGACAATTTGTGATTTCACAAAACCAAGAGGCTATAAAGGAGCTAGTCTCC	
	OmpA_GD_AF269261	(658)	GAAATGTTGAATGTAACTTCCAGCCGACGACAATTTGTGTTTCACAACCCCTAGAGGATACAAGGGAACGTCGCGC	
	OmpA_NJ1_AF269266	(655)	GAAATGCTCAATGTAACTTCAAGCCGACGACAATTTGTGATTTCACAAAACCAAGAGGCTATAAAGGAACTGGCTCG	
	PCR Omp1_D_92/1293	(661)	GAAATGCTCAATGTAACTTCAAGCCGACGACAATTTGTGATTTCACAAAACCAAGAGGCTATAAAGGAACTGGCTCG	
	OmpA_MN-VR122-Cal10_AF269262	(622)	GAAATGCTCAACGTCACTTCAAGCCGACGACAATTTGTGATTTCACAAAACCAAGAGGCTATAAAGGAGCTAGTCTCG	
	OmpA_VS225_AF269259	(649)	GAAATGCTGAATGTAACTTCCAGCCCAAACACAATTTGTAGTTTCACAAAACCAAGAGGCTATAAAGGAAACAGGATCG	
	OmpA_WSRTE30_AY762613	(682)	GAAATGCTCAACGTCACTTCAAGCCGACGACAATTTGTGATTTCACAAAACCAAGAGGCTATAAAGGAGCTAGTCTCG	
	OmpA_M56_AF269268	(682)	GAAATGCTCAACGTAACTTCAAGCCGACGACAATTTGTGATTTCACAAAACCAAGAGGCTATAAAGGAAACAGGATCG	
	OmpA_WC_AF269269	(658)	GAAATGCTTAATGTAACTTCTAGCCGACGACAATTTGTAAATTCACAAAACCTAGGGGATATAAAGGAAACAACCTCT	
	Consensus	(751)	GAAATGCT AATGTAACTTC AGCCGACGACAATTTGTGATTTCACAAAACCTAGAGGCTATAAAGGAAAC G CTCG	900
	OmpA_Ctra_NC000117_CT681_RC	(740)	----TTCCCTTTGATCTTACAGCAGGAAACAGATGCTCGCACAGGAACCTAAAGGATGCCTCTATTGATTACCATGAA	
	OmpA_Cmur_AE002160_TC0052_RC	(731)	----TTCCCTTTAAACATAAAGCTTGAACAGTGTAGGCTACAGATCTAAAGATGCTTCCATGATACCATGAG	
	OmpA_Cppn_NC000922_CPn0695	(731)	----TCCCCTTTACACAGACGCTGGCTAGCAACAGCTACTGGAACAAAGTCTGCGACCATCAATATCATGAA	
	OmpA_Cpfel_NC007899_CF0958	(736)	AACITCCCCTTACCTGTAGCAGCTGGCACAGCAACTGCAACAGATATAAATCAGCTACTGTTAATGATACCATGAA	
	OmpA_Cpcav_AE015925_CCA00047_RC	(727)	AACITCCCCCTGCTCTTAACCGCTTGAACAGAGGCTACTGATFACATAAATCAGCTCAAAATTAAGTATCATGAA	
	OmpA_Cpabo_NC004552_CAB048_RC	(731)	----TTCCCTTTA C TCTTAACAGCTGGTACTGATCAGGCAACTGACATAAGTCGGTCAAAATTAATACCACGAA	
	OmpA_84-2334_AJ310735	(733)	AACITTTCTTTACCCGAAACAACTGGTTCTGATGCTGTCTACAGATACTAAATCTGCAACACTCAATATCATGAA	
	OmpA_6BC_M73035	(823)	AATTTTCCTTTACCTATAAACGGCTGGAACCAACAGAAGCTACAGACACCAAAATCAGTCAAAATTAATACCATGAA	
	OmpA_CP3_AF269265	(757)	AATTTTCCTTTACCTATACCGCTTGGAAACCAACAGAAGCTACAGACACCAAAATCAGTCAAAATTAATACCATGAA	
	OmpA_GD_AF269261	(733)	AACITTTCTTTACCTGTCAAAATGGCGACAGAGGCTCTACGGATACTAAATCTGCAACACTCAATATCATGAA	
	OmpA_NJ1_AF269266	(730)	AATTTTCCTTTACCTATAGACGCGGTACAGAGGCTCTACAGATFACAAAGTCTGCAACACTCAATATCATGAA	
	PCR Omp1_D_92/1293	(736)	AATTTTCCTTTACCTATAGACGCGGTACAGAGGCTCTACAGATFACAAAGTCTGCAACACTCAATATCATGAA	
	OmpA_MN-VR122-Cal10_AF269262	(697)	AATTTTCCTTTACCTATAACCGCTTGGAAACCAACAGAAGCTACAGACACCAAAATCAGTCAAAATTAATACCATGAA	
	OmpA_VS225_AF269259	(724)	AACITTCCTTTACCTCTAACAGCTGTACAGATGGTGTCTACAGATFACAAATCTGCAACACTCAATATCATGAA	
	OmpA_WSRTE30_AY762613	(757)	AATTTTCCTTTACCTATAACCGGTGGAAACCAACAGAAGCTACAGACACCAAAATCAGTCAAAATTAATACCATGAA	
	OmpA_M56_AF269268	(757)	AATTTTCCTTTACCTATAACCGGTGGTACTGATGTCGACAGACACCAAAATCAGTCAAAATTAATACCATGAA	
	OmpA_WC_AF269269	(733)	AATTTTCCTTTACCTCTAACGCTGSCACACACTGCTACAGATFACAAAGTCAAGTCAAGTCAAAATTAATATCATGAA	
	Consensus	(826)	AA TTTCCCTTTACCTATAAC GCTGG ACAGA G TGCTACAGATACTAAATC GCTACAAATTAATACCATGAA	

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Figure 4 – continued (6)

OmpA_Ctra_NC000117_CT681_RC	(811)	901	TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	975
OmpA_Cmur_AE002160_TC0052_RC	(802)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_Cppn_NC000922_CPn0695	(802)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_CpfeI_NC007899_CF0958	(811)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_Cpcav_AE015925_CCA00047_RC	(802)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_Cpabo_NC004552_CAB048_RC	(802)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_84-2334_AJ310735	(808)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_6BC_M73035	(898)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_CP3_AF269265	(832)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_GD_AF269261	(808)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_NJ1_AF269266	(805)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
PCR Omp1_D_92/1293	(811)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_MN-VR122-Cal10_AF269262	(772)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_VS225_AF269259	(799)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_WSRTE30_AY762613	(832)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_M56_AF269268	(832)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
OmpA_WC_AF269269	(808)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	
Consensus	(901)		TGGCAAGTGGTCTAGCTCTCTCTTACAGACTGAATATGTTCACTCCCTACATGGAGTTAAATGGTCTCAGACA	1050
OmpA_Ctra_NC000117_CT681_RC	(886)	976	AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_Cmur_AE002160_TC0052_RC	(877)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_Cppn_NC000922_CPn0695	(877)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_CpfeI_NC007899_CF0958	(886)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_Cpcav_AE015925_CCA00047_RC	(877)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_Cpabo_NC004552_CAB048_RC	(877)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_84-2334_AJ310735	(883)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_6BC_M73035	(973)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_CP3_AF269265	(907)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_GD_AF269261	(883)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_NJ1_AF269266	(880)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
PCR Omp1_D_92/1293	(886)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_MN-VR122-Cal10_AF269262	(847)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_VS225_AF269259	(874)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_WSRTE30_AY762613	(907)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_M56_AF269268	(907)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
OmpA_WC_AF269269	(883)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	
Consensus	(976)		AGCTTTGATGCCGATACGATTGCTATAGCCAGCCAAAATCAGCTACAGCTATTTTGTACTACCACGCTTAAC	

Figure 4 – continued (7)

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OmpA_Ctra_NC000117_CT681_RC	(961)	1051	1125
OmpA_Cmur_AE002160_TC0052_RC	(952)		
OmpA_Cppn_NC000922_CPn0695	(952)		
OmpA_CpfeI_NC007899_CF0958	(961)		
OmpA_Cpcav_AE015925_CCA00047_RC	(952)		
OmpA_Cpabo_NC004552_CAB048_RC	(952)		
OmpA_84-2334_AJ310735	(958)		
OmpA_6BC_M73035	(1048)		
OmpA_CP3_AF269265	(982)		
OmpA_GD_AF269261	(958)		
OmpA_NJ1_AF269266	(955)		
PCR Omp1_D_92/1293	(961)		
OmpA_MN-VR122-Cal10_AF269262	(922)		
OmpA_VS225_AF269259	(949)		
OmpA_WSRTE30_AY762613	(982)		
OmpA_M56_AF269268	(982)		
OmpA_WC_AF269269	(958)		
OmpA_WC_AF269269	(1051)		
Consensus			
OmpA_Ctra_NC000117_CT681_RC	(1030)	1126	1200
OmpA_Cmur_AE002160_TC0052_RC	(1012)		
OmpA_Cppn_NC000922_CPn0695	(1018)		
OmpA_CpfeI_NC007899_CF0958	(1027)		
OmpA_Cpcav_AE015925_CCA00047_RC	(1018)		
OmpA_Cpabo_NC004552_CAB048_RC	(1018)		
OmpA_84-2334_AJ310735	(1024)		
OmpA_6BC_M73035	(1123)		
OmpA_CP3_AF269265	(1057)		
OmpA_GD_AF269261	(1024)		
OmpA_NJ1_AF269266	(1021)		
PCR Omp1_D_92/1293	(1027)		
OmpA_MN-VR122-Cal10_AF269262	(997)		
OmpA_VS225_AF269259	(1015)		
OmpA_WSRTE30_AY762613	(1057)		
OmpA_M56_AF269268	(1057)		
OmpA_WC_AF269269	(1024)		
Consensus	(1126)		

Figure 4 – continued (8)

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OmpA_Ctra_NC000117_CT681_RC	(1105)	1201	GACAAATACGCAGTTACAGTTGAGACTCGCTTGATCGATGAGAGAGCAGCTCACGTAATGCACAAATCCCGCTTC	1275
OmpA_Cmur_AE002160_TC0052_RC	(1087)		GATAAATATGCAAGTTACTGTTGAGACACGCTTGATCGATGAAAGAGCAGCTCACGTAATGCAGTTCCGCTTC	
OmpA_Cppn_NC000922_CPn0695	(1093)		GATAAATGGTCACTTACTGCGAAGCTCGTTTAATTAACGAGAGAGCTGCTCACGTATCTCGTCAATTCAGATTC	
OmpA_CpfeI_NC007899_CF0958	(1102)		GATAAATGGTCCATTACTGGTGAAGCAGCTTAATCGACGAAAGAGCTGCTCACATTAATGCTCAATTCAGATTC	
OmpA_Cpcav_AE015925_CCA00047_RC	(1093)		GACAAATGGTCCGATCACTGGTGAAGCTCGCTTAATCAACGAAAGAGCTGCTCACGTAACGCTCAATTCAGATTC	
OmpA_Cpabo_NC004552_CAB048_RC	(1093)		GACAAATGGTCAATCACTGGTGAAGCAGCTTAATCAATGAAGAGCCGCTCACATGAATGCTCAATTCAGATTC	
OmpA_84-2334_AJ310735	(1090)		-----	
OmpA_6BC_M73035	(1198)		GACAAATGGTCAATCACTGGTGAAGCAGCTTAATCAATGAAGAGCTGCTCACATGAATGCTCAATTCAGATTC	
OmpA_CP3_AF269265	(1132)		GACAAATGGTCAATCACTGGTGAAGCAGCTTAATCAATGAAGAGCTGCTCACATGAATGCTCAATTCAGATTC	
OmpA_GD_AF269261	(1099)		GACAAATGGTCAATCACTGGTGAAGCAGCTTAATCAATGAAGAGCTGCTCACATGAATGCTCAATTCAGATTC	
OmpA_NJ1_AF269266	(1096)		GACAAATGGTCAATCACTGGTGAAGCAGCTTAATCCATGAAGAGCTGCTCACATGAATGCTCAATTCAGATTC	
PCR Omp1_D_92/1293	(1069)		-----	
OmpA_MN-VR122-Cal10_AF269262	(1072)		GACAAATGGTCAATCACTGGTGAAGCAGCTTAATCAATGAAGAGCTGCTCACATGAATGCTCAATTCAGATTC	
OmpA_VS225_AF269259	(1090)		GACAAATGGTCAATCACTGGTGAAGCAGCTTAATCAATGAAGAGCTGCTCACATGAATGCTCAATTCAGATTC	
OmpA_WSRTE30_AY762613	(1099)		-----	
OmpA_M56_AF269268	(1132)		GATAAATGGTCCATCACTGGTGAAGCAGCTTAATCAATGAAGAGCTGCTCACATGAATGCTCAATTCAGATTC	
OmpA_WC_AF269269	(1099)		GACAAATGGTCAATCACTGGTGAAGCAGCTTAATCAATGAAGAGCTGCTCACATGAATGCTCAATTCAGATTC	
OmpA_WC_AF269269	(1201)		GACAAATGGTCAATCACTGGTGAAGCAGCTTAATCAATGAAGAGCTGCTCACATGAATGCTCAATTCAGATTC	1350
Consensus			-----	
OmpA_Ctra_NC000117_CT681_RC	(1180)		1276	
OmpA_Cmur_AE002160_TC0052_RC	(1162)		TAA-----	
OmpA_Cppn_NC000922_CPn0695	(1168)		TAA-----	
OmpA_CpfeI_NC007899_CF0958	(1177)		TAA-----	
OmpA_Cpcav_AE015925_CCA00047_RC	(1168)		TAA-----	
OmpA_Cpabo_NC004552_CAB048_RC	(1168)		TAA-----	
OmpA_84-2334_AJ310735	(1090)		-----	
OmpA_6BC_M73035	(1273)		TAAGGATTTAGTTT-A-----	
OmpA_CP3_AF269265	(1207)		TAAGGATTTAGTTT-ATACATCCTAACTTTTGTCCCGCTATCAGAACCTGGGAGTCTCCGGGTTCTGATTTT	
OmpA_GD_AF269261	(1174)		TAAGGATTTAGTTT-ATACATCCTAACTTTTGTCCCGCTATCAGAACCTGGGAGTCTCCGGGTTCTGATTTT	
OmpA_NJ1_AF269266	(1171)		TAAGGATTTAGTTG-ATACGTCTTAACCTTTTGTCCCGCTATCAGAACCTGGGAGTCTCCGGGTTCTGATTTT	
PCR Omp1_D_92/1293	(1069)		-----	
OmpA_MN-VR122-Cal10_AF269262	(1147)		TAAGGATTTAGTTT-ATACATCCTAACTTTTGTCCCGCTATCAGAACCTGGGAGTCTCCGGGTTCTGATTTT	
OmpA_VS225_AF269259	(1165)		TAAGGATTTAGTTT-ATACATCCTAACTTTTGTCCCGCTATCAGAACCTGGGAGTCTCCGGGTTCTGATTTT	
OmpA_WSRTE30_AY762613	(1099)		-----	
OmpA_M56_AF269268	(1207)		TAAGGATTTAGTTTATACATCCTAACTTTTGTCCCGCTATCAGAACCTGGGAGTCTCCGGGTTCTGATTTT	
OmpA_WC_AF269269	(1174)		TAAGGATTTAGTTT-ATACATCCTAACTTTTGTCCCGCTATCAGAACCTGGGAGTCTCCGGGTTCTGATTTT	
Consensus	(1276)		TAA	

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Figure 4 – continued (9)

OmpA_Ctra_NC000117_CT681_RC	(1183)	1351	1425
OmpA_Cmur_AE002160_TC0052_RC	(1165)	---	---
OmpA_Cppn_NC000922_CPn0695	(1171)	---	---
OmpA_CpfeI_NC007899_CF0958	(1180)	---	---
OmpA_Cpcav_AE015925_CCA00047_RC	(1171)	---	---
OmpA_Cpabo_NC004552_CAB048_RC	(1171)	---	---
OmpA_84-2334_AJ310735	(1090)	---	---
OmpA_6BC_M73035	(1288)	---	---
OmpA_CP3_AF269265	(1281)	TTTGCTACCAACCCCTTTTCAGA-GTTTCAAAATCTCTTTTCTAAAAATCCGTTGCGATCAGAAATTCACGTGATTATCTA	---
OmpA_GD_AF269261	(1248)	TTTGCTACATCCTTTTGTATAGCTTAATACCTCTTTTAAAAATCCATTGCGACAAAGAAATTCACGTGATTATCTA	---
OmpA_NJ1_AF269266	(1245)	TTTGCTACCAACCCCTTTT-CAGAGTTTCAAAATCTCTTTTCTAAAAATCCATTGCGATCAGAGTTCAAGTCAAGTATCTA	---
PCR Omp1_D_92/1293	(1069)	---	---
OmpA_MN-VR122-Cal10_AF269262	(1221)	TTTGCTACCAACCCCTTTTCAGA-GTTTCAAAATCTCTTTTCTAAAAATCCGTTGCGATCAGAAATTCACGTGATTATCTA	---
OmpA_VS225_AF269259	(1239)	TTTGCTACCAACCCCTTTTGCAGAGTTTCAAAATCTCTTTTCTAAAAATCCGTTGCGATTAAGAAATTCACGTGATTATCTA	---
OmpA_WSRTE30_AY762613	(1099)	---	---
OmpA_M56_AF269268	(1282)	TTTGCCGCGATTCTTTTGTAGAGTTTCAAAATCTCTTTTCTAAAAATCCATTGCGATTAAGAAATTCACGTGATTATCTA	---
OmpA_WC_AF269269	(1248)	TTTGCTACCAACCCCTTTTGCAGAGTTTCAAAATCTCTTTTCTAAAAATCCGTTGCGATTAAGAAATTCACGTGATTATCTA	---
Consensus	(1351)	1426	1500
OmpA_Ctra_NC000117_CT681_RC	(1183)	---	---
OmpA_Cmur_AE002160_TC0052_RC	(1165)	---	---
OmpA_Cppn_NC000922_CPn0695	(1171)	---	---
OmpA_CpfeI_NC007899_CF0958	(1180)	---	---
OmpA_Cpcav_AE015925_CCA00047_RC	(1171)	---	---
OmpA_Cpabo_NC004552_CAB048_RC	(1171)	---	---
OmpA_84-2334_AJ310735	(1090)	---	---
OmpA_6BC_M73035	(1288)	---	---
OmpA_CP3_AF269265	(1355)	AAATTTTCTAGAAGCTAGAAACCTAGAGATTACAATCTTGGCTAAAAAGCATTTATTAATATATC-TCTCTATTCT	---
OmpA_GD_AF269261	(1323)	AAATTTTCTAGAAGCTTGAACCTAGAGATTACAACCTTGGCTAAAAAGCATTTATTAACATAACATCTCTATTCT	---
OmpA_NJ1_AF269266	(1319)	AAATTTTCTAGAAGCTAGAAACCTAGAGATTACAATCTTGGCTAAAAAGCATTTATTAATTAACATCTCTATTCT	---
PCR Omp1_D_92/1293	(1069)	---	---
OmpA_MN-VR122-Cal10_AF269262	(1295)	AAATTTTCTAGAAGCTAGAAACCTAGAGATTACAATCTTGGCTAAAAAGCATTTATTAATTAACATCTCTATTCT	---
OmpA_VS225_AF269259	(1314)	AAATTTTCTAGAAGCTAGAAACCTAGAGATTACAATCTTGGCTAAAAAGCATTTATTAATTAACATCTCTATTCT	---
OmpA_WSRTE30_AY762613	(1099)	---	---
OmpA_M56_AF269268	(1357)	AAATTTTCTAGAAGCTTAAACCTAGAGATTACAACCTTGGCTAAAAAGCATTTATTAACATAACATCTCTATTCT	---
OmpA_WC_AF269269	(1323)	AAATTTTCTAGAAGCTAGAAACCTAGAGATTACAATCTTGGCTAAAAAGCATTTATTAATTAACATCTCTATTCT	---
Consensus	(1426)	---	---

Figure 4 – continued (10)

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OmpA_Ctra_NC000117_CT681_RC	(1183)	1501	1537	(SEQ ID 123)
OmpA_Cmur_AE002160_TC0052_RC	(1165)	----	----	(SEQ ID 124)
OmpA_Cppn_NC000922_CPn0695	(1171)	----	----	(SEQ ID 125)
OmpA_CpfeI_NC007899_CF0958	(1180)	----	----	(SEQ ID 126)
OmpA_Cpcav_AE015925_CCA00047_RC	(1171)	----	----	(SEQ ID 127)
OmpA_Cpabo_NC004552_CAB048_RC	(1171)	----	----	(SEQ ID 128)
OmpA_84-2334_AJ310735	(1090)	----	----	(SEQ ID 129)
OmpA_6BC_M73035	(1288)	----	----	(SEQ ID 130)
OmpA_CP3_AF269265	(1429)	----	----	(SEQ ID 131)
OmpA_GD_AF269261	(1398)	----	----	(SEQ ID 132)
OmpA_NJ1_AF269266	(1394)	----	----	(SEQ ID 133)
PCR Omp1_D_92/1293	(1069)	----	----	(SEQ ID 134)
OmpA_MN-VR122-Cal10_AF269262	(1370)	----	----	(SEQ ID 135)
OmpA_VS225_AF269259	(1389)	----	----	(SEQ ID 136)
OmpA_WSRTE30_AY762613	(1099)	----	----	(SEQ ID 137)
OmpA_M56_AF269268	(1432)	----	----	(SEQ ID 138)
OmpA_WC_AF269269	(1398)	----	----	(SEQ ID 139)
Consensus	(1501)	----	----	(SEQ ID 140)

Figure 4 – continued (11)

Major outer membrane protein (MOMP)	sequences	1	75
OmpA_Ctra_NC000117_CT681	(1)	-----MKLLKSVLVFAALS-SASSLQALPVGNPAEPSLIMIDGILWEGFGGDPDPCA	
OmpA_Cmur_AE002160_TC0052	(1)	-----MKLLKSVLFAVLG-SASSLHALPVGNPAEPSLIMIDGILWEGFGGDPDCT	
OmpA_Cppn_NC000922_CPn0695	(1)	-----MKLLKSALLSAAFAGSVGSLQALPVGNPSDFSLLIDGTIWEGAAGDPDPCA	
OmpA_Cpfe1_NC007899_CF0958	(1)	-----MKLLKSALLFAAAG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCA	
OmpA_Cpcav_AE015925_CCA00047	(1)	-----MKLLKSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCS	
OmpA_Cpabo_NC004552_CAB048	(1)	-----MKLLKSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCS	
OmpA_84-2334_AJ310735	(1)	-----MKLLKSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCA	
OmpA_6BC_M73035	(1)	RRAN-NSEHKKRY-A-SLEVSMKLLKSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCA	
OmpA_CP3_AF269265	(1)	-----MKLLKSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCA	
OmpA_GD_AF269261	(1)	-----MKLLKSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCS	
OmpA_NJ1_AF269266	(1)	-----KLLKSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCA	
PCR Omp1 D 92/1293	(1)	-----MKLLKSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCA	
OmpA_MN-VR122-Ca110_AF269262	(1)	-----QALPVGNPAEPSLLIDGTWEGASGDPDPCA	
OmpA_VS225_AF269259	(1)	-----LLKSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCA	
OmpA_WSRTE30_AY762613	(1)	-----MKLLKSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCA	
OmpA_M56_AF269268	(1)	-----MKLLKSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCS	
OmpA_WC_AF269269	(1)	-----KSALLFAATG-SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCA	
Consensus	(1)	-----MKLLKSALLFAATG SALSQALPVGNPAEPSLLIDGTWEGASGDPDPCA	150
OmpA_Ctra_NC000117_CT681	(53)	TWCDAlSMRVGYGDFVDFRVLKTDVNKEFQ-MGAKPTTDG--NSAAPSTLTARENPAAYGRHMQDAEMFTNAAC	
OmpA_Cmur_AE002160_TC0052	(53)	TWCDAlSLRLGYGDFVDFRVLKTDVNKQFE-MGAAPTGDAD--LTAPTASR-ENPAYGKHMQDAEMFTNAAY	
OmpA_Cppn_NC000922_CPn0695	(54)	TWCDAlSRAGFYGDYVDFRILKVDAPKTFs-MGAKPTGSA---AANYTTAVDRPNPAYNKHHLDAEWFTNAGF	
OmpA_Cpfe1_NC007899_CF0958	(53)	TWCDAlSIRAGFYGDYVDFRILKVDNKTISGMAAAPTAAASGTASNTTV--AADRSNFAYGKHLQDAEWCTNAAY	
OmpA_Cpcav_AE015925_CCA00047	(53)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTIS-MGTAPTGNA---AADEKTVADRNNIAYGKHMQDAEWSTNAAF	
OmpA_Cpabo_NC004552_CAB048	(53)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTITMGAVPTGTA---AANYKTPTDRPNIAYGKHLQDAEWFTNAAF	
OmpA_84-2334_AJ310735	(53)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTFTMGAVPTGNS---AADFKTPTDRPNIAYGKHLQDAEWFTNAAF	
OmpA_6BC_M73035	(72)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTFTSGMAATPTQATGNASNTNQPEANGRPNIAYGRHMQDAEWFsNAAF	
OmpA_CP3_AF269265	(53)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTFTSGMAATPTQATGNASNTNQPEANGRPNIAYGRHMQDAEWFsNAAF	
OmpA_GD_AF269261	(53)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTFTSGIKKPTGSS---PNDFKNAEDRPNVAYGRHLQDSEWFTNAAF	
OmpA_NJ1_AF269266	(51)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTFTSGMAKSPTTEATGTASATTT--AVDRTNLAYGKHLQDAEWFTNAAF	
PCR Omp1 D 92/1293	(53)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTFTSGMAKSPTTEATGTASATTT--AVDRTNLAYGKHLQDAEWFTNAAF	
OmpA_MN-VR122-Ca110_AF269262	(33)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTFTSGMAATPTQATGNASNTNQPEANGRPNIAYGRHMQDAEWFsNAAF	
OmpA_VS225_AF269259	(50)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTISGMAAPTQSA---AADYKTPTDRPNIAYGKHLQDAEWFTNAAF	
OmpA_WSRTE30_AY762613	(53)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTFTSGMAATPTQATGNASNTNQPEANGRPNIAYGRHMQDAEWFsNAAF	
OmpA_M56_AF269268	(53)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTFTSGMAATPTTEASGNATNTGTPEANGRPNIAYGRHMQDAEWFsNAAF	
OmpA_WC_AF269269	(48)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTFTSGMAAIPTESSGTVSSAKQ--AVDRNLAYGKHLQDAEWFTNSAF	
Consensus	(76)	TWCDAlSIRAGYGYGDYVDFRILKVDNKTFTSGMAA PT ATG ASAT A DR NIAYGKHLQDAEWFTNAAF	

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Figure 4 – continued (12)

151	OmpA_Ctra_NC000117_CT681	(125)	MALNIWDRFDVFCITLGATSGYLKGNASAFNLVGLFGDNEQ--KTVKAESVPNMMSFDQSVVELYTDTTFAWSVGA	226
	OmpA_Cmur_AE002160_TC0052	(124)	MALNIWDRFDVFCITLGATSGYLKGNASAFNLVGLFGRDET----AAVADDIPNVSLSQAVVELYTDTTFAWSVGA	
	OmpA_Cppn_NC000922_CPn0695	(124)	IALNIWDRFDVFCITLGASNGYIRGNSTAFNLVGLFGVKG-----TTVNANELPNVLSNGVVELYTDTTFSWSVGA	
	OmpA_CpfeI_NC007899_CF0958	(126)	LALNIWDRFDVFCITLGASNGYFKASSDAFNLVGLIGLAG-----TDFANQRPNVEISQGLVELYTDTTFSWSVGA	
	OmpA_Cpcav_AE015925_CCA00047	(123)	LALNIWDRFDVFCITLGASNGYLKANAFAFNLVGLIGVTG-----TDLQGQYPNVAISQGLVELYTDTTFSWSVGA	
	OmpA_Cpabo_NC004552_CAB048	(124)	LALNIWDRFDVFCITLGASNGYFKASSAFNLVGLIGVKG-----SIAADQLPNVIGITQGVVEFYTDTTFSWSVGA	
	OmpA_84-2334_AJ310735	(124)	LALNIWDRFDVFCITLGASNGYFKASSAFNLVGLIGIKGN-----TLTNDRLPNVIGITQGVVEFYTDTTFSWSVGA	
	OmpA_6BC_M73035	(147)	LALNIWDRFDVFCITLGASNGYFKASSAFNLVGLIGFSAASSISITDLPMQLPNVIGITQGVVEFYTDTSFSWSVGA	
	OmpA_CP3_AF269265	(128)	LALNIWDRFDVFCITLGASNGYFKSSAAAFNLVGLIGFSATNSTDLPMQLPNVIGITQGVVEFYTDTSFSWSVGA	
	OmpA_GD_AF269261	(124)	LALNIWDRFDVFCITLGASNGYFKSSAAAFNLVGLIGVKG-----SLTNDQLPNVAITQGVVEFYTDTTFSWSVGA	
	OmpA_NJ1_AF269266	(124)	LALNIWDRFDVFCITLGASNGYFKASSAFNLVGLIGLKG-----TDFNNQLPNVAITQGVVEFYTDTTFSWSVGA	
	PCR_Omp1_D_92/1293	(126)	LALNIWDRFDVFCITLGASNGYFKASSAFNLVGLIGLKG-----TDFNNQLPNVAITQGVVEFYTDTTFSWSVGA	
	OmpA_MN-VR122-Cal10_AF269262	(108)	LALNIWDRFDVFCITLGASNGYFKSSAAAFNLVGLIGFSATSTSTELPMQLPNVIGITQGVVEFYTDTSFSWSVGA	
	OmpA_VS225_AF269259	(121)	LALNIWDRFDVFCITLGASNGYFKASSAFNLVGLIGVKG-----SVAADQLPNVIGITQGVVEFYTDTTFSWSVGA	
	151	OmpA_WSRTF30_AY762613	(128)	
OmpA_M56_AF269268		(128)	LALNIWDRFDVFCITLGASNGYFKSSAAAFNLVGLIGFSASSAVSTDLPKQLPNVAITQGVVEFYTDTSFSWSVGA	
OmpA_WC_AF269269		(121)	LALNIWDRFDVFCITLGASNGYFKSSAAAFNLVGLIGIAGN-SESNAINDQLPNVAITQGVVEFYTDTTFSWSVGA	
Consensus		(151)	LALNIWDRFDVFCITLGASNGYFKASSAFNLVGLIGV G TD QLPNVAITQGVVEFYTDTTFSWSVGA	
OmpA_Ctra_NC000117_CT681		(198)	RAALWECGCATLGASFQYAQSKPKVEELNVLCHAAEFTINKPKGVGK--EFLPLDTAGTDAATGCTKDSIDYHE	
OmpA_Cmur_AE002160_TC0052		(195)	RAALWECGCATLGASFQYAQSKPKVEELNVLCHAAEFTINKPKGVGQ--EFLPLNKAAGTVSATDTKDSIDYHE	
OmpA_Cppn_NC000922_CPn0695		(195)	RGALWECGCATLGAEFFQYAQSKPKVEELNVCNVVSQFSVKNPKGYKGA--FPLPLTDAGVATATGKTSATINYHE	
OmpA_CpfeI_NC007899_CF0958		(196)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVTSSPAQFMIHKPRGYKGTAANFPLPVAAGTATATDKSATVKYHE	
OmpA_Cpcav_AE015925_CCA00047		(193)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVISSPTQFVIHKPRGYKGTAANFPLPLTAGTESATDKSATIKYHE	
OmpA_Cpabo_NC004552_CAB048		(195)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVSSPAQFVWHKPRGYKGT--AFPLPLTAGTDQATDKSATIKYHE	
OmpA_84-2334_AJ310735		(195)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVTSSPAQFVWHKPRGYKGATANFSLPETTGSDAATDKSATLKYHE	
OmpA_6BC_M73035		(222)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVTSSPAQFVIHKPRGYKGASSNFPLPITAGTTEATDKSATIKYHE	
OmpA_CP3_AF269265		(203)	RGALWECGCATLGAEFFQYAQSNPKIEMIINVTSSPAQFVIHKPRGYKGASSNFPLPITAGTTEATDKSATIKYHE	
OmpA_GD_AF269261		(195)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVISSPAQFVWHKPRGYKGTSANFPLPANAGTEAATDKSATLKYHE	
151		OmpA_NJ1_AF269266	(194)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVTSSPAQFVIHKPRGYKGTSNFPLPIDAGTEAATDKSATLKYHE
	PCR_Omp1_D_92/1293	(196)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVTSSPAQFVIHKPRGYKGTSNFPLPIDAGTEAATDKSATLKYHE	
	OmpA_MN-VR122-Cal10_AF269262	(183)	RGALWECGCATLGAEFFQYAQSNPKIEMIINVTSSPAQFVIHKPRGYKGASSNFPLPITAGTTEATDKSATIKYHE	
	OmpA_VS225_AF269259	(192)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVISSPTQFVWHKPRGYKGTSNFPLPITAGTDGATDKSATLKYHE	
	OmpA_WSRTF30_AY762613	(203)	RGALWECGCATLGAEFFQYAQSNPKIEMIINVTSSPAQFVIHKPRGYKGASSNFPLPITAGTTEATDKSATIKYHE	
	OmpA_M56_AF269268	(203)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVTSSPAQFVIHKPRGYKGTSNFPLPITAGTDDATDKSATIKYHE	
	OmpA_WC_AF269269	(195)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVTSSPAQFVIHKPRGYKGTTSNFPLPITAGTDATDKSATIKYHE	
	Consensus	(226)	RGALWECGCATLGAEFFQYAQSNPKIEMNLNVTSSPAQFVIHKPRGYKGTSNFPLPITAGTD ATDKSATIKYHE	

Figure 4 – continued (13) 17/23

OmpA_Ctra_NC000117_CT681	(271)	WQASLALSYRLNMF	301	375
OmpA_Cmur_AE002160_TC0052	(268)	WQASLALSYRLNMF		
OmpA_Cppn_NC000922_CPn0695	(268)	WQASLALSYRLNMF		
OmpA_Cpfe1_NC007899_CF0958	(271)	WQGLALSYRLNMF		
OmpA_Cpcav_AE015925_CCA00047	(268)	WQIGLALSYRLNMF		
OmpA_Cpabo_NC004552_CAB048	(268)	WQVGLALSYRLNMF		
OmpA_84-2334_AJ310735	(270)	WQVGLALSYRLNMF		
OmpA_6BC_M73035	(297)	WQVGLALSYRLNMF		
OmpA_CP3_AF269265	(278)	WQVGLALSYRLNMF		
OmpA_GD_AF269261	(270)	WQVGLALSYRLNMF		
OmpA_NJ1_AF269266	(269)	WQVGLALSYRLNMF		
PCR Omp1_D_92/1293	(271)	WQVGLALSYRLNMF		
OmpA_MN-VR122-Cal10_AF269262	(258)	WQVGLALSYRLNMF		
OmpA_VS225_AF269259	(267)	WQVGLALSYRLNMF		
OmpA_WSRTE30_AY762613	(278)	WQVGLALSYRLNMF		
OmpA_M56_AF269268	(278)	WQVGLALSYRLNMF		
OmpA_WC_AF269269	(270)	WQVGLALSYRLNMF		
Consensus	(301)	WQVGLALSYRLNMF	376	450
OmpA_Ctra_NC000117_CT681	(344)	VSLQINKMKSRKSCG		
OmpA_Cmur_AE002160_TC0052	(338)	VSLQINKMKSRKSCG		
OmpA_Cppn_NC000922_CPn0695	(340)	VSCQINKFKSRKACG		
OmpA_Cpfe1_NC007899_CF0958	(343)	VSMQINKMKSRKACG		
OmpA_Cpcav_AE015925_CCA00047	(340)	ASLQINKMKSRKACG		
OmpA_Cpabo_NC004552_CAB048	(340)	ASIQINKMKSRKACG		
OmpA_84-2334_AJ310735	(342)	ASIQINKMKSRKACG		
OmpA_6BC_M73035	(372)	ASIQINKMKSRKACG		
OmpA_CP3_AF269265	(353)	ASIQINKMKSRKSCG		
OmpA_GD_AF269261	(342)	ASIQINKMKSRKACG		
OmpA_NJ1_AF269266	(341)	ASIQINKMKSRKACG		
PCR Omp1_D_92/1293	(343)	ASIQINKMKSRKAC		
OmpA_MN-VR122-Cal10_AF269262	(333)	ASIQINKMKSRKACG		
OmpA_VS225_AF269259	(339)	ASIQINKMKSRKACG		
OmpA_WSRTE30_AY762613	(353)	ASIQINKMKSRKAC		
OmpA_M56_AF269268	(353)	ASIQINKMKSRKSCG		
OmpA_WC_AF269269	(342)	ASIQINKMKSRKACG		
Consensus	(376)	ASIQINKMKSRKACG		

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Figure 4 – continued (14)

OmpA_Ctra_NC000117_CT681	(394)	-----	507	(SEQ ID 141)
OmpA_Cmur_AE002160_TC0052	(388)	-----		(SEQ ID 142)
OmpA_Cppn_NC000922_CPn0695	(390)	-----		(SEQ ID 143)
OmpA_CpfeI_NC007899_CF0958	(393)	-----		(SEQ ID 144)
OmpA_Cpcav_AE015925_CCA00047	(390)	-----		(SEQ ID 145)
OmpA_Cpabo_NC004552_CAB048	(390)	-----		(SEQ ID 146)
OmpA_84-2334_AJ310735	(364)	-----		(SEQ ID 147)
OmpA_6BC_M73035	(426)	-----		(SEQ ID 148)
OmpA_CP3_AF269265	(426)	LLPFSEFQISFLKSVRIRH-LSKIF-KLET-RLQSCVKSIKLSLYS-HAPV---		(SEQ ID 149)
OmpA_GD_AF269261	(415)	LLTSFCIA-YLFFKIHSHKNSLII-NFLEA-NLEITTLRKKHY-TNISILSTRPYSM		(SEQ ID 150)
OmpA_NJ1_AF269266	(391)	-----		(SEQ ID 151)
PCR Omp1_D_92/1293	(357)	-----		(SEQ ID 152)
OmpA_MN-VR122-Cal10_AF269262	(383)	-----		(SEQ ID 153)
OmpA_VS225_AF269259	(389)	-----		(SEQ ID 154)
OmpA_WSRTE30_AY762613	(367)	-----		(SEQ ID 155)
OmpA_M56_AF269268	(403)	-----		(SEQ ID 156)
OmpA_WC_AF269269	(392)	-----		(SEQ ID 157)
Consensus	(451)	-----		(SEQ ID 158)

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Figure 5

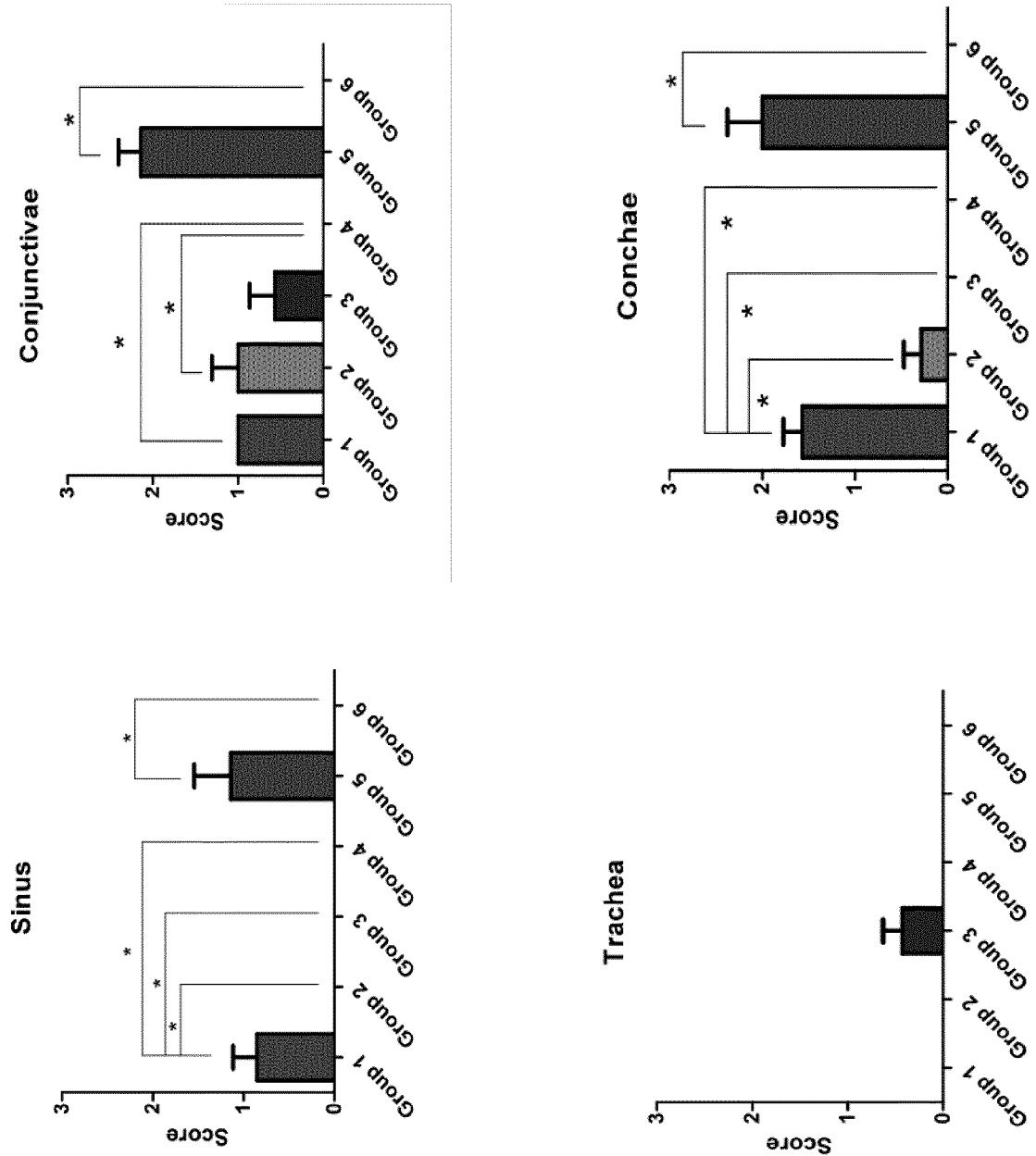


Figure 5 – continued (1)

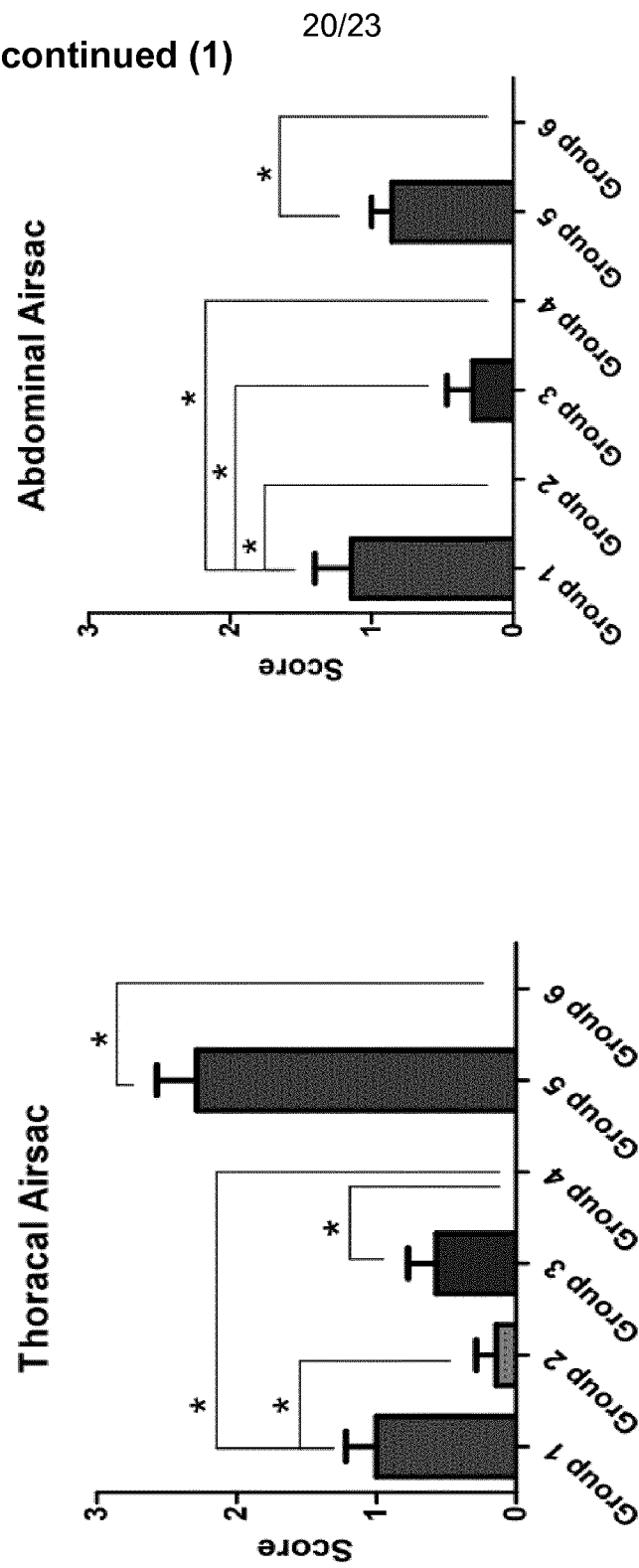


Figure 5 – continued (2) 21/23

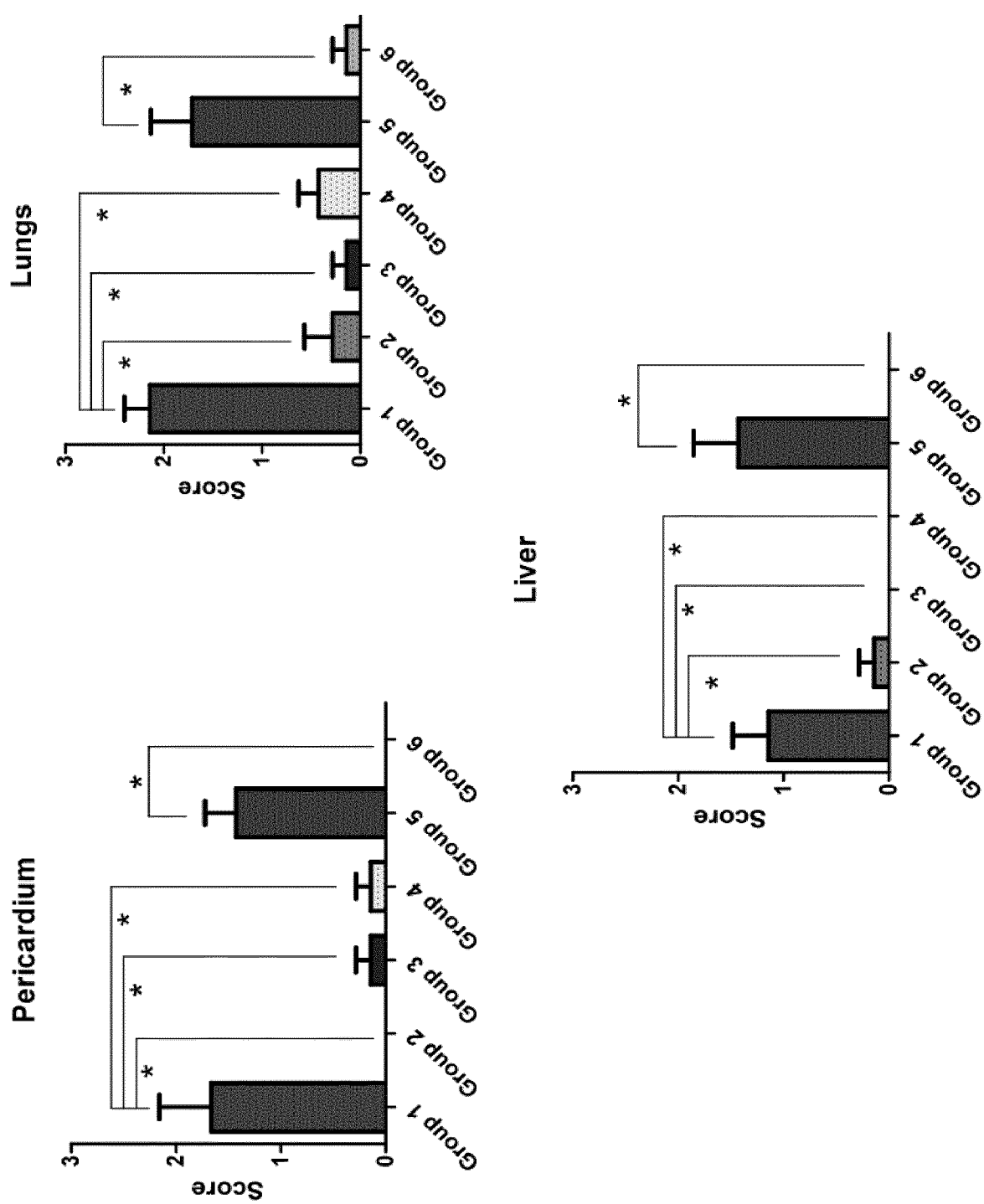


Figure 6

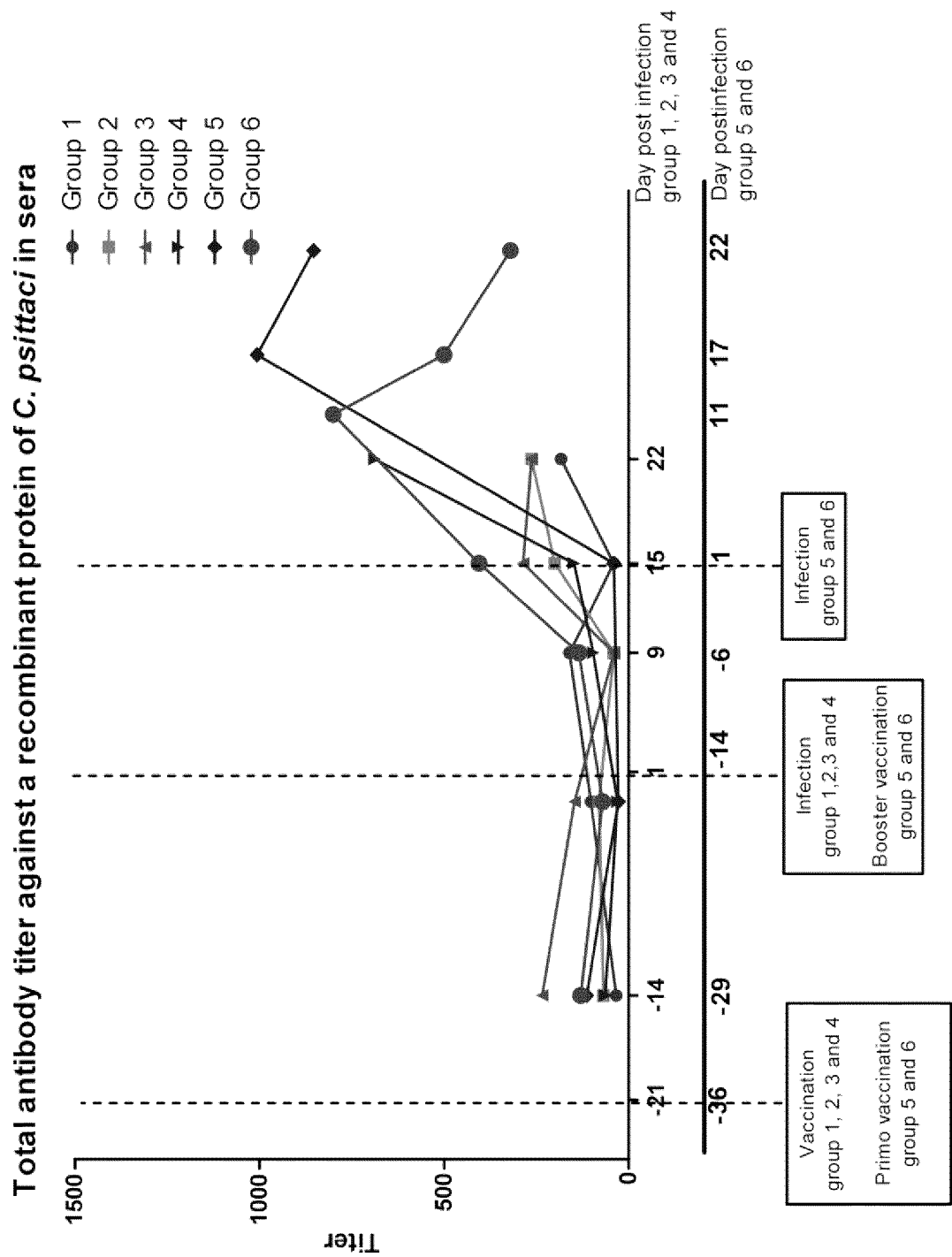
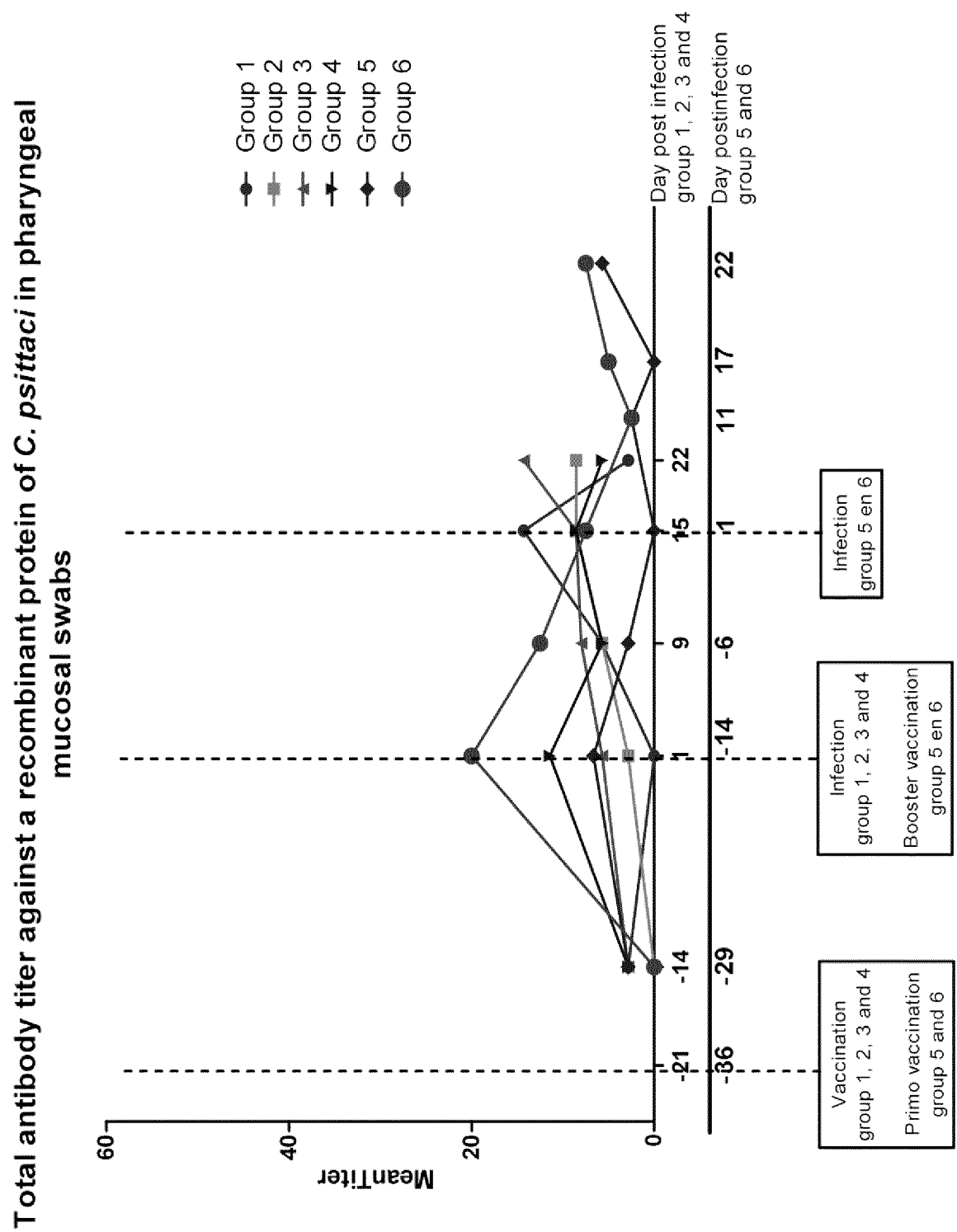


Figure 7



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/061420

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K38/10 A61K39/118 A61P31/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 August 2012

Date of mailing of the international search report

31/08/2012

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Ferreira, Roger

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2012/061420

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International application No

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International application No

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International application No

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