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(54) Title: POLYNUCLEOTIDES AND POLYPEPTIDES FOR THE DETECTION OF RICKETTSIA HELVETICA

(57) Abstract: The present invention relates to newly identified polynucleotides and polypeptides encoded by such polynucleotides for detecting and/or monitoring the presence of Rickettsia helvetica. More particularly, the present invention relates to polynucleotides encoding an OmpA polypeptide of Rickettsia helvetica and antigenic fragments thereof and the respective polypeptides and antigenic fragments as well as to methods for detecting and/or monitoring the presence of Rickettsia helvetica in a subject and respective kits.



WO 2007/113009 A2

Polynucleotides and polypeptides for the detection of *Rickettsia helvetica*

Field of the invention

The present invention relates to newly identified polynucleotides and polypeptides encoded by
5 such polynucleotides for detecting and/or monitoring the presence of *Rickettsia helvetica*.
More particularly, the present invention relates to polynucleotides encoding an OmpA
polypeptide of *Rickettsia helvetica* and antigenic fragments thereof and the respective
polypeptides and antigenic fragments as well as to methods for detecting and/or monitoring
the presence of *Rickettsia helvetica* in a subject and respective kits.

Background of the invention

Rickettsiae are well known gram-negative pathogenic bacteria. The genus *Rickettsia* is
considered to consist of three groups of strictly intracellular bacteria, namely the typhus (TG),
the spotted fever (SFG) and the scrub typhus (STG) groups. The SFG *Rickettsiae* are mainly
15 transmitted by ticks and at least 17 different species of SFG have been described so far
(Bouyer et al., 2001, Int. J. Syst. Evol. Microbiol. 51, 339-347).

The species definition of SFG has been based on their serotype as determined by e. g.
microimmunofluorescence test (MIF) and cross-adsorption. *Rickettsia helvetica* (*R. helvetica*),
which belongs to the SFG group, has been isolated from the tick *Ixodes ricinus* collected in
20 Switzerland, France, Slovenia and Sweden (Fournier et al., 2000, Emerging Infectious
Diseases, 6, 389-392). Infection of *R. helvetica* in humans can cause severe clinical symptoms
such as prolonged fever, nausea, headache and myalgia, whereas evidence for human *R.*
helvetica infections has been found so far in Europe, Thailand and possibly Australia or Japan
(Phongmany et al., 2006, Emerging Infectious Diseases, 12, 256-262).

25 The usual method for the diagnosis of rickettsioses is serologic testing, whereas
microimmunofluorescence assays, detecting antibodies to one or more rickettsial antigens are
mostly used. However, serological cross-reactions are common among the SFG *Rickettsiae*,
which makes it difficult and costly to discriminate different species of the SFG group with
serological methods. WO 99/42479 describes the use of an antigenic amino acid sequence
30 from the 17 kDa outer membrane protein of *R. helvetica* for serological testing of an infected
patient. Unfortunately, the 17 kDa outer membrane protein is very conserved among
Rickettsiae and the sequence used by the applicant of WO 99/42479, has 100 % identity to the

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corresponding sequence of the 17 kDa outer membrane protein of *R. sibirica* (Genbank Accession number: AAO38436), *R. conorii* (Genbank Accession number: AAL03825), *R. japonica* (Genbank Accession number: Q52764) and *R. rickettsii* (Genbank Accession number: ABA01551), which makes it difficult to discriminate *R. sibirica*, *R. conorii*, *R. rickettsii* or *R. japonica* from *R. helvetica* using this sequence. Apart from the 17 kDa outer membrane protein, other proteins and/or the respective genes have been used to identify Rickettsiae using serological testing or PCR amplification such as 16S rRNA, citrate synthase, the envelope proteins OmpA and OmpB (for review see La Scola and Raoult, 1997, J. Clin. Microbiol., 35, 2715-2727) or the PS120 protein encoding gene D as described by Sekeyova et al., 2001, Int. J. Syst. Evol. Microbiol. 51, 1353-1360.

Most of these genes are not divergent enough, however, to distinguish Rickettsiae on a species-specific level. A fragment of the *ompA* gene was used to identify strains of different SFG group Rickettsiae, using PCR-restriction fragment length polymorphism (Roux et al., 1996, J. Clin. Microbiol., 34, 2058-2065). A 632-bp region at the 5' end of the gene was amplified by these authors and an amplification product was obtained for most of the strains except for DNA from *R. akari*, *R. australis*, *R. helvetica* and *R. belli*.

In view of the fact that *R. helvetica* is a frequent human pathogen as can be seen from the occurrence of *R. helvetica* infections described above, there is a need of the identification of new tools and methods which allow for highly specific detection of *R. helvetica* and reliable discrimination of this pathogenic bacteria from other Rickettsiae of the SFG group.

Summary of the invention

The object of the present invention is therefore to provide new tools and methods which allow for highly specific detection of *R. helvetica* using serological as well as polynucleotide amplification procedures and which reliably discriminate this pathogen bacteria from other Rickettsiae. This object and other objects as will be apparent from the following description have been achieved by providing new polynucleotides, polypeptides and antigenic fragments thereof and new primers and probes which can be used for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject. Surprisingly, it has been found that *R. helvetica* harbours a gene encoding an OmpA polypeptide which has not been identified yet and which contains various antigenic fragments specific for *R. helvetica*.

Thus, the present invention provides a polynucleotide encoding an OmpA polypeptide of *Rickettsia helvetica* selected from the group consisting of:

- a) polynucleotides encoding the OmpA polypeptide of *Rickettsia helvetica* comprising the amino acid sequence as shown in SEQ ID NO: 1;
- 5 b) polynucleotides comprising the nucleotide sequence as shown in SEQ ID NO: 2;
- c) polynucleotides the complementary strand of which hybridizes under stringent conditions to a polynucleotide as defined in (a) or (b);
- d) polynucleotides which are at least 80 % identical to a polynucleotide as defined in (a) or (b).

10

Further provided is a polynucleotide encoding an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* selected from the group consisting of:

- a) polynucleotides encoding an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* comprising at least 5 consecutive amino acids located from amino acid 1 to amino
- 15 acid 800 of the amino acid sequence as shown in SEQ ID NO: 1;
- b) polynucleotides comprising a nucleotide sequence selected from SEQ ID NOS: 3-17;
- c) polynucleotides which are at least 60 % identical to a polynucleotide comprising a nucleotide sequence selected from SEQ ID NOS: 3-17.
- d) polynucleotides which are at least 90 % identical to a polynucleotide comprising a
- 20 nucleotide sequence selected from SEQ ID NOS: 3-17.

Further provided is an OmpA polypeptide of *Rickettsia helvetica* having the amino acid sequence encoded by the polynucleotide encoding an OmpA polypeptide of *Rickettsia helvetica* as defined above as well as an OmpA polypeptide of *Rickettsia helvetica* selected

25 from the group consisting of:

- a) polypeptides comprising the amino acid sequence as shown in SEQ ID NO: 1;
- b) polypeptides comprising an amino acid sequence which is at least 80 % identical in amino acid sequence to the amino acid sequence as defined in (a).

30 As well provided is an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* having the amino acid sequence encoded by the polynucleotide encoding an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* as defined above and an antigenic

fragment of an OmpA polypeptide of *Rickettsia helvetica* selected from the group consisting of

a) an antigenic fragment comprising at least 5 consecutive amino acids located from amino acid 1 to amino acid 800 of the amino acid sequence as shown in SEQ ID NO: 1;

5 b) an antigenic fragment comprising an amino acid sequence selected from SEQ ID NOS: 18-32;

c) an antigenic fragment comprising an amino acid sequence which is at least 60 % identical to an amino acid sequence selected from SEQ ID NOS: 18-32;

10 d) an antigenic fragment comprising an amino acid sequence which is at least 80 % identical to an amino acid sequence selected from SEQ ID NOS: 18-32;

e) an antigenic fragment consisting of at least 5 consecutive amino acids of an amino acid sequence selected from SEQ ID NOS: 18-32.

15 A further object is to provide an antibody against the polynucleotides, the OmpA polypeptides or the antigenic fragments of the present invention as defined above, for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject as well as a method for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject, comprising assaying a biological fluid of the subject with a compound selected from the polynucleotides, the polypeptides, the antigenic fragments and the antibody as well as with the primers and/or
20 probes of the present invention and a respective diagnostic kit. Further provided is a vector comprising at least one copy of the polynucleotides of the present invention as well as a prokaryotic host cell transformed with such polynucleotides and/or such vector as well as primers for amplifying DNA encoding an OmpA polypeptide of *Rickettsia helvetica*.

25 Other objects and advantages will become apparent to those skilled in the art from a review of the ensuing detailed description.

Detailed description of the invention

30 As used herein, the following definitions are supplied in order to facilitate the understanding of the present invention.

As used herein, the terms “polypeptide”, “peptide”, “protein”, “polypeptidic” and “peptidic” are used interchangeably to designate a series of amino acid sequence connected to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent sequence.

5 “Antigenic fragments of amino acids sequences” or “antigenic fragments of a polypeptide” or “antigenic fragments thereof” refer to an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the respective sequence of the polypeptide from which they derive and which has antigenic properties. An antigenic fragment will generally include at least about 5, preferably between 5 and 100, most preferably between 10 and 50, in
10 particular between 10 and 30 or more consecutive amino acid residues of the full-length molecule, that comprise an epitope, provided that the fragment in question retains immunoreactivity in the assays described herein, i.e. the fragment binds an antibody produced in a subject in response to the presence of *R. helvetica*. The term “epitope” as used herein refers to a sequence of at least about 3 to 5, preferably about 5 to 10 or 15 amino acids, which
15 define a sequence that by itself or as part of a larger sequence, binds to an antibody generated in response to such sequence. There is no critical upper limit to the length of the fragment, which may comprise nearly the full-length of the protein sequence, or even a fusion protein comprising two or more epitopes. An epitope for use in the present invention is not limited to a polypeptide having the exact sequence of the portion of the parent protein from which it is
20 derived. Useful antigenic fragments are designed using computer-assisted analysis of amino acid sequences which identify probable surface exposed, antigenic regions.

“Species specific antigenicity” refers to the antigenicity of an antigenic fragment of a polypeptide which is specific to a particular bacteria species such that the polypeptide or the
25 antigenic fragment does not cross-react with other species of the same genus of the bacteria, i. e. shows no genus specific antigenicity. Species specific antigenicity can be determined e. g. after cross-absorption by a number of methods, including e. g. Western blot, ELISA (enzyme-linked immunosorbent assays, equivalent used herein to Enzyme Immunoassay (EIA)) or MIF (Micro-Immunofluorescence). Usually, antigenic fragments used in the methods of the present
30 invention are selected as to show species specific antigenicity, i. e. the antigenic fragment is species specific to *R. helvetica*.

Conservative amino acid substitutions are herein defined as exchanges for example within one of the following five groups:

- I. Small aliphatic, nonpolar or slightly polar sequence: Ala, Ser, Thr, Pro, Gly
- II. Polar, positively charged sequence: His, Arg, Lys
- 5 III. Polar, negatively charged sequence: and their amides: Asp, Asn, Glu, Gln
- IV. Large, aromatic sequence: Phe, Tyr, Trp
- V. Large, aliphatic, nonpolar sequence: Met, Leu, Ile, Val, Cys.

As herein used, the term "stringent conditions" means hybridization will occur between
10 nucleotide sequences only if there is at least 95% and preferably at least 97% identity between the sequences.

A "vector" a "vector expressible in a host" or "expression vector" is a polynucleotide construct, generated recombinantly or synthetically, with a series of specified polynucleotide
15 elements that permit transcription of a particular nucleotide sequence in a host cell. Typically, this vector includes a transcriptional unit comprising a particular nucleotide sequence to be transcribed operably linked to a promoter. A vector expressible in a host can be e. g. an autonomously or self-replicating plasmid, a cosmid or a phage.

20 The terms "host", "host cell" and "recombinant host cell" are used interchangeably herein to indicate a prokaryotic or eukaryotic cell into which one or more vectors or isolated and purified nucleotide sequences of the invention have been introduced. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to
25 either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

The term "identity", "sequence identity" or "identical to" refers to the number of exactly matching residues (expressed as a percentage) in a sequence alignment between the template
30 sequence and the sequence relative. Identity of polynucleotide sequences or amino acid sequences can be measured using sequence analysis software known to the person skilled in

the art such as e. g. the BLAST program of the National Center for Biotechnology Information, Bethesda, USA (<http://www.ncbi.nlm.nih.gov>).

The term "cell transfected" or "cell transformed" or "transfected/transformed cell" means the cell into which an extracellular nucleotide sequence has been introduced and thus harbors the extracellular nucleotide sequence. The nucleotide sequence might be introduced into the cell so that the nucleotide is replicable either as a chromosomal integrant or as an extra chromosomal element.

"Antibody" refers to a class of plasma proteins produced by the B-cells of the immune system after stimulation by an antigen. Mammal (i.e. human) antibodies are immunoglobulins of the Ig G, M, A, E or D isotype. The term "antibody" as used for the purposes of this invention includes, but is not limited to, polyclonal antibodies, monoclonal antibodies, anti-idiotypic antibodies, as well as chimeric antibodies. The term antibody is used to mean whole antibodies and binding fragments thereof. Binding fragments include single chain fragments, Fv fragments and Fab fragments.

As is known in the art, an allelic variant is an alternate form of a polypeptide that is characterized as having a substitution, deletion, addition or repeat of one or more amino acids that does not alter the biological function of the polypeptide. By "biological function" is meant the function of the polypeptide in the cells in which it naturally occurs, even if the function is not necessary for the growth or survival of the cells. For example, the biological function of a porin is to allow the entry into cells of compounds present in the extracellular medium. Biological function is distinct from antigenic property. A polypeptide can have more than one biological function. Allelic variants are very common in nature. For example, a bacterial species is usually represented by a variety of strains that differ from each other by minor allelic variations. Indeed, a polypeptide that fulfills the same biological function in different strains can have an amino acid sequence (and polynucleotide sequence) that is not identical in each of the strains as can be seen e. g. from Ryffel et al. (Ryffel et al., 2003, BMC Infectious Diseases, 3:14) who demonstrated that the average identity of the deduced amino acid sequences of the OspA proteins from different strains of *Borrelia valaisiana* is about 69,9 %. In view of this fact, the polypeptides of the present invention include naturally-occurring

allelic variants, including mutants or any other non-naturally occurring variants or fragments thereof that retain the inherent characteristics of the respective polypeptide such as species specific antigenicity of at least one antigenic fragment of the polypeptide.

- 5 The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA, preferably it is in the form of DNA. The DNA may be doublestranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The polynucleotide of the present invention may encode for a polypeptide, or for a polypeptide having a signal sequence. The
- 10 polypeptides, their antigenic fragments or variants thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies.

- The nucleotide sequence of polynucleotides as defined herein encoding a polypeptide of
- 15 *Rickettsia helvetica* or an antigenic fragment of that polypeptide may be as well a different sequence which sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide or the same antigenic fragment of that polypeptide.

- The polynucleotides and polypeptides of the present invention are preferably provided in an
- 20 isolated form, and preferably are purified to homogeneity. The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides
- 25 could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

- In a first aspect the present invention provides a polynucleotide encoding an OmpA
- 30 polypeptide of *Rickettsia helvetica* selected from the group consisting of:
- a) polynucleotides encoding the OmpA polypeptide of *Rickettsia helvetica* comprising the amino acid sequence as shown in SEQ ID NO: 1;

- b) polynucleotides comprising the nucleotide sequence as shown in SEQ ID NO: 2;
c) polynucleotides the complementary strand of which hybridizes under stringent conditions to a polynucleotide as defined in (a) or (b);
d) polynucleotides which are at least 80 % identical to a polynucleotide as defined in (a) or (b).

Polynucleotides as defined in c) the complementary strand of which hybridizes under stringent conditions to a polynucleotide as defined in (a) or (b) are polynucleotides of which hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

Polynucleotides as defined in d) are preferably at least 85 %, more preferably at least 90 %, most preferably at least 95 %, in particular at least 97 %, most particular at least 99 % identical to a polynucleotide as defined in (a) or (b).

In a further aspect the invention provides a polynucleotide encoding an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* selected from the group consisting of:

- a) polynucleotides encoding an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* comprising at least 5 consecutive amino acids located from amino acid 1 to amino acid 800 of the amino acid sequence as shown in SEQ ID NO: 1;
b) polynucleotides comprising a nucleotide sequence selected from SEQ ID NOS: 3-17;
c) polynucleotides which are at least 60 % identical to a polynucleotide comprising a nucleotide sequence selected from SEQ ID NOS: 3-17.
d) polynucleotides which are at least 90 % identical to a polynucleotide comprising a nucleotide sequence selected from SEQ ID NOS: 3-17.

Preferably, polynucleotides as defined in a) encode an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* which comprises at least 10 consecutive amino acids located from amino acid 1 to amino acid 800 of the amino acid sequence as shown in SEQ ID NO: 1. More preferably, polynucleotides as defined in a) encode an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* which comprises between 5 and 100, most preferably between 10 and 50, in particular between 10 and 30 consecutive amino acids located from amino acid 1 to amino acid 800 of the amino acid sequence as shown in SEQ ID

NO: 1.

Preferably, polynucleotides as defined in a) encode an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* comprising at least 5, usually at least 10, preferably
5 between 5 and 100, most preferably between 10 and 50, in particular between 10 and 30 consecutive amino acids, located from amino acid 1 to amino acid 150 and/or from amino acid 151 to amino acid 350 and/or from amino acid 351 to amino acid 550 and/or from amino acid 551 to amino acid 800 of the amino acid sequence as shown in SEQ ID No 1. More preferably, polynucleotides as defined in a) encode an antigenic fragment of an OmpA
10 polypeptide of *Rickettsia helvetica* comprising at least 5, usually at least 10, preferably between 5 and 100, most preferably between 10 and 50, in particular between 10 and 30 consecutive amino acids consecutive amino acids located from about amino acid 49 to about amino acid 100 and/or from about amino acid 173 to about amino acid 309 and/or from about amino acid 393 to about amino acid 521 and/or from about amino acid 597 to about amino
15 acid 776 of the amino acid sequence as shown in SEQ ID No 1. Most preferred are polynucleotides as defined in a) which encode an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* comprising at least 5, preferably at least 10 consecutive amino acids located from about amino acid 87 to about amino acid 100 and/or from about amino acid 194 to about amino acid 218 and/or from about amino acid 295 to about amino acid 309 and/or
20 from about amino acid 406 to about amino acid 420 and/or from about amino acid 494 to about amino acid 518 and/or from about amino acid 507 to about amino acid 521 and/or from about amino acid 657 to about amino acid 675 and/or from about amino acid 697 to about amino acid 711 and/or from about amino acid 758 to about amino acid 776 of the amino acid sequence as shown in SEQ ID No 1.

25 The preferred polynucleotides of the polynucleotides as defined in b) comprise a nucleotide sequence selected from SEQ ID NOS: 3-13, in particular the nucleotide sequences which are identical to each other but are located on different positions of the polynucleotide such as SEQ ID NOS: 3 and 4 and SEQ ID NOS: 9 and 10, respectively. More preferred polynucleotides of
30 the polynucleotides as defined in b) comprise a nucleotide sequence selected from SEQ ID NOS: 3-11. More particular preferred polynucleotides of the polynucleotides as defined in b) comprise a nucleotide sequence selected from SEQ ID NOS: 3-8. Most particular preferred

polynucleotides of the polynucleotides as defined in b) comprise a nucleotide sequence selected from SEQ ID NOS: 3, 4, 6, 8 and 11.

Polynucleotides as defined in c) are preferably at least 70 %, more preferably at least 80 % most preferably at least 90 %, in particular at least 95 % identical to a polynucleotide as

5 defined in (b), whereas the nucleotide sequence is preferably selected from SEQ ID NOS: 3, 4, 6, 8, 10, 11, 12, 14, and 17 . Polynucleotides as defined in d) are preferably at least 95 % identical to a polynucleotide as defined in (b), whereas the nucleotide sequence is preferably selected from SEQ ID NOS: 5, 7, 9, 13, 15, and 16.

10 Polynucleotides of *R. helvetica* are usually found in ticks, in particular in *Ixodes ricinus* ticks. The polynucleotides of this invention were isolated from *Ixodes ricinus* ticks. Usually the ticks are collected e. g. using the close dragging method and DNA is isolated from the collected ticks by methods known to the person skilled in the art. On the basis of the isolated DNA, the identification of the presence of *R. helvetica* from the collected ticks can be performed by

15 known methods such as e. g. PCR using the gene coding for the intracytoplasmatic PS 120 protein as described in Sekeyova et al., 2001, Int. J. Syst. Evol. Microbiol. 51, 1353-1360

In a further aspect the present invention provides an OmpA polypeptide of *Rickettsia helvetica* having the amino acid sequence encoded by the polynucleotide encoding an OmpA

20 polypeptide of *Rickettsia helvetica* as defined above. Usually, such a polypeptide is at least 80 %, preferably at least 85 %, and more preferably at least 90, most preferably at least 95 %, in particular at least 97 %, most particular at least 99 % identical to the amino acid shown in SEQ ID NO: 1.

25 The present invention provides as well an OmpA polypeptide of *Rickettsia helvetica* selected from the group consisting of

- a) polypeptides comprising the amino acid sequence as shown in SEQ ID NO: 1;
- b) polypeptides comprising an amino acid sequence which is at least 80 % identical in amino acid sequence to the amino acid sequence as defined in (a).

30

Polypeptides as defined in b) comprise preferably an amino acid sequence which is at least 85 %, more preferably at least 90%, most preferably at least 95%, in particular at least 97 %, in particular at least 99 % identical to the amino acid sequence as defined in (a).

most particular at least 99 % identical in amino acid sequence to the amino acid sequence as defined in (a) and that preferably differs from the sequence of reference by conservative amino acid substitutions which do not destroy the species specific antigenicity of the polypeptide.

- 5 An OmpA polypeptide of *Rickettsia helvetica* provided by the present invention include naturally-occurring allelic variants, including mutants or any other non-naturally occurring variants or fragments thereof that retain the inherent characteristics of the OmpA polypeptide of *Rickettsia helvetica* such as species specific antigenicity of at least one antigenic fragment of the polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 1.

10

A further object of the present invention is to provide antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* selected from the group consisting of

- a) an antigenic fragment comprising at least 5 consecutive amino acids located from amino acid 1 to amino acid 800 of the amino acid sequence as shown in SEQ ID NO: 1;
- 15 b) an antigenic fragment comprising an amino acid sequence selected from SEQ ID NOS: 18-32;
- c) an antigenic fragment comprising an amino acid sequence which is at least 60 % identical to an amino acid sequence selected from SEQ ID NOS: 18-32;
- d) an antigenic fragment comprising an amino acid sequence which is at least 80 % identical
- 20 to an amino acid sequence selected from SEQ ID NOS: 18-32;
- e) an antigenic fragment consisting of at least 5 consecutive amino acids of an amino acid sequence selected from SEQ ID NOS: 18-32.

An antigenic fragment as defined in a) comprises preferably at least 10 consecutive amino acids located from amino acid 1 to amino acid 800 of the amino acid sequence as shown in SEQ ID NO: 1. More preferably, an antigenic fragment as defined in a) comprises between 5 and 100, most preferably between 10 and 50, in particular between 10 and 30 consecutive amino acids located from amino acid 1 to amino acid 800 of the amino acid sequence as shown in SEQ ID NO: 1.

30

Preferably, an antigenic fragment as defined in a) comprises at least 5, usually at least 10, preferably between 5 and 100, most preferably between 10 and 50, in particular between 10

and 30 consecutive amino acids located from amino acid 1 to amino acid 150 and/or from amino acid 151 to amino acid 350 and/or from amino acid 351 to amino acid 550 and/or from amino acid 551 to amino acid 800 of the amino acid sequence as shown in SEQ ID No 1.

More preferably, an antigenic fragment as defined in a) comprises at least 5, usually at least

5 10, preferably between 5 and 100, most preferably between 10 and 50, in particular between 10 and 30 consecutive amino acids located from about amino acid 49 to about amino acid 100 and/or from about amino acid 173 to about amino acid 309 and/or from about amino acid 393 to about amino acid 521 and/or from about amino acid 597 to about amino acid 776 of the amino acid sequence as shown in SEQ ID NO: 1. Most preferred is an antigenic fragment as
10 defined in a) which comprises at least 5, preferably at least 10 consecutive amino acids located from about amino acid 87 to about amino acid 100 and/or from about amino acid 194 to about amino acid 218 and/or from about amino acid 295 to about amino acid 309 and/or from about amino acid 406 to about amino acid 420 and/or from about amino acid 494 to about amino acid 518 and/or from about amino acid 507 to about amino acid 521 and/or from about amino
15 acid 657 to about amino acid 675 and/or from about amino acid 697 to about amino acid 711 and/or from about amino acid 758 to about amino acid 776 of the amino acid sequence as shown in SEQ ID No 1.

Preferred antigenic fragments as defined in b) comprise an amino acid sequence selected from
20 SEQ ID NOS: 18-28 in particular the amino acid sequences which are identical to each other but are located on different positions of the polypeptide such as SEQ ID NOS: 18 and 19 and SEQ ID NOS: 24 and 25, respectively. More particular preferred antigenic fragments as defined in b) comprise an amino acid sequence selected from SEQ ID NOS: 18-27 and 30-32.

Antigenic fragments as defined in c) are preferably at least 70 %, more preferably at least
25 80 %, most preferably at least 90 %, in particular at least 95 % identical to the amino acid sequences as defined in (b), whereas the amino acid sequence is preferably selected from SEQ ID NOS: 18-27 and 30-32. Antigenic fragments as defined in d) are preferably at least 90 %, in particular at least 95 % identical to the amino acid sequences as defined in (b), whereas the amino acid sequence is preferably selected from SEQ ID NOS: 28, 29.

30 Most particular preferred antigenic fragments as defined in b), c), d) and e), comprise antigenic fragments, whereas the amino acid sequence is selected from SEQ ID NOS: 18, 19, 21, 23 and 26. The antigenic fragment as defined in (e), consists preferably of at least 10

consecutive amino acids of an amino acid sequence selected from SEQ ID NOS: 18-32

The antigenic fragments of an OmpA polypeptide of *Rickettsia helvetica* of the present invention are selected, such as that they retain their inherent characteristics such as species
5 specific antigenicity. The antigenic fragment may recombinantly produced or may be produced by chemical synthesis.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide. The polypeptide of the present invention is preferably a
10 recombinant polypeptide produced by using a polynucleotide of the present invention. The polypeptide may comprise additional amino acids which are fused to the polypeptide, such as a signal sequence or a sequence which is employed for purification of the polypeptide.

The present invention further provides an antibody against the polynucleotides, the OmpA
15 polypeptides or the antigenic fragments thereof as defined above for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject. The preferred antibody of the present invention is an antibody against the OmpA polypeptides or the antigenic fragments as defined above.

Antibodies of the present invention can be polyclonal antibodies, monoclonal antibodies, anti-
20 idiotypic antibodies, as well as chimeric antibodies, preferably monoclonal antibodies.

Various procedures known in the art may be used for the production of such antibodies and fragments. Antibodies generated against the polypeptides and the antigenic fragments of the present invention can be obtained by direct injection of the polypeptides or the antigenic fragments into an animal or by administering them to an animal, preferably a nonhuman. In
25 this manner, even a sequence encoding an antigenic fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to detect or to isolate the polypeptide or its antigenic fragments from tissue or a biological fluid containing that polypeptide.

A more preferred antibody of the present invention is a monoclonal antibody against an
30 antigenic fragment of an OmpA polypeptide of the present invention, in particular a monoclonal antibody against an antigenic fragment comprising an amino acid sequence selected from SEQ ID NOS: 18-32.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBVhybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

A further aspect of the present invention is to provide a method for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject, comprising assaying a biological fluid of the subject with a compound selected from

- a) a polynucleotide encoding an OmpA polypeptide of *Rickettsia helvetica* as defined above;
- b) a polynucleotide encoding an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* as defined above;
- c) an OmpA polypeptide of *Rickettsia helvetica* as defined above;
- d) an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* as defined above;
- e) an antibody against the polynucleotides, the OmpA polypeptides or the antigenic fragments of an OmpA polypeptide of *Rickettsia helvetica* as defined above.

Procedures for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject according to the method of the invention by assaying a biological fluid of a subject may be conventional, and will admit of design and selection by the skilled person who understands the present disclosure, mindful of well accepted principles of diagnostic and immunodiagnostic methodology and interpretation. Such principles are set forth, for example, in Harlow, E., and Lane, D., "Antibodies: A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 349 (1988), Rose, Noel R., et al., eds., Manual of Clinical Laboratory Immunology, Fifth Edition, ASM Press, Washington D. C. (1997), and prior editions ; Rich, R. et al., eds. , Clinical Immunology, Mosby, Publisher, St. Louis (1995).

Typical examples of assays which can utilize the polynucleotides of the invention are polynucleotide amplification procedures which permit amplification of a target polynucleotide such as polymerase chain reaction (PCR), nested PCR or real time PCR. The polynucleotides of the invention can be used as a basis to select probes and/or primers for amplification which

are specific for a target DNA of *R. helevtica* encoding an OmpA polypeptide or an antigenic fragment of an OmpA polypeptide. Particluar useful to amplify DNA of *R. helevtica* are the primers comprising the sequences SEQ ID NO: 33 (forward primer) and SEQ ID NO: 34 (reverse primer), which are encompassed by the present invention.

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Typical examples of assays which can utilize the polypeptides, the antigenic fragments thereof and the respective antibodies of the invention are serological assays like enzyme-linked immunosorbent assays (ELISA), immunofluorescence assays (IFA), Western immunoblot assays, cross-absorption assays. The types of assays which can be used by practising the method of the present invention and which can be incorporated in kit form are many, and include, for example, indirect assays or direct assays, with indirect assays presently preferred as well as competitive and non-competitive assays, with non-competitive assays presently preferred. These kinds of assays comprise as well as immunometric or sandwich immunoassays. By the term "immunometric assay" or "sandwich immunoassay," it is meant to include simultaneous sandwich, forward sandwich and reverse sandwich immunoassays. These terms are well understood by those skilled in the art. Those of skill will also appreciate that, the polypeptides, the antigenic fragments thereof and the respective antibodies of the invention will be useful in other variations and forms of assays which are presently known or which may be developed in the future. These are intended to be included within the scope of the present invention. Preferably, the method is a serological assay selected from enzyme-linked immunosorbent assays (ELISA), immunofluorescence assays (IFA), Western immunoblot assays and cross-absorption assays and, wherein the biological fluid of the subject is assayed with a compound selected from the polypeptides, the antigenic fragments thereof and the respective antibodies of the invention as defined above. More preferably the serological assay is an ELISA and the compound used is an antigenic fragment of the present invention. Antibodies produced in relation to the presence of *Rickettsia helvetica* in a subject which can be detected and/or monitored by the method of the invention are of the isotypes IgG, IgA and IgM. As biological fluid, usually diluted serum is used, wheras serum samples are diluted between 1:5 and 1:1000, preferably between 1:10 and 1:500, more preferably between 1:10 and 1:100. Assays of this type used for monitoring and/or detecting *Rickettsiae* are summarised e. g. in La Scola and Raoult, 1997, J. Clin. Microbiol., 35, 2715-2727.

30

In case the serological assay is an ELISA and a solid support is used, which is preferred, the

polypeptides, the antigenic fragments thereof and the respective antibodies of the invention can be either covalently or physically bound to that solid support, by techniques such as covalent bonding via an amide, ester or disulfide linkage, or by adsorption. Those skilled in the art will know many other suitable solid support immunoadsorbents and methods for immobilizing polypeptides, antigenic fragments thereof and antibodies thereon. The polypeptides, the antigenic fragments thereof and the respective antibodies of the invention can be bound directly to the solid support or can be bound via a binding pair such as the biotin/streptavidin system. In case antigenic fragments are used, they are preferably biotin labelled, whereas a spacer, such as amino hexanoic acid, might be introduced between the fragment and biotin. There are many solid supports which can be used as immunoadsorbents for the method and the kit of the present invention. Well known materials which may be employed include glass, polystyrene, polypropylenes dextran, nylon, agarose, dextran, acrylamide, Nitrocellulose, PVDF and other materials, in the form of tubes, beads, membranes and microtiter plates formed from or coated with such materials, and the like.

Another object of the present invention is to provide a diagnostic kit for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject, comprising a compound selected from

- a) a polynucleotide encoding an OmpA polypeptide of *Rickettsia helvetica* as defined above;
 - b) a polynucleotide encoding antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* as defined above;
 - c) an OmpA polypeptide of *Rickettsia helvetica* as defined above;
 - d) an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* as defined above;
 - e) an antibody against the polynucleotides, the OmpA polypeptides or the antigenic fragments of an OmpA polypeptide of *Rickettsia helvetica* as defined above;
- optionally with reagents and/or instructions for use.

Such a kit may comprise further labels and labelled compounds known to the skilled person. Such a kit may comprise as well a carrier means being compartmentalized to receive in close confinement therewith one or more container means such as plates, vials, tubes and the like, each of said container means comprising the separate elements of the assay to be used. The kit of the present invention may further comprise reagents for carrying out an assay as described

above. Preferably, the kit includes reagents for carrying out an ELISA, more preferably a non-competitive ELISA, most preferably an indirect non-competitive ELISA. Such reagents are well known to the person skilled in the art and comprise e. g. Immobilization-buffer, Blocking-buffer, Incubation-buffer, Wash-buffer, Substrate-buffer and/or Secondary-Antibody-Conjugate.

The subject as referred herein is usually a human or an animal, preferably a human. The method as well as the kit can be applied to a biological fluid of a subject such as serum or endothelial cells of blood vessels. Usually, the biological fluid is obtained as a sample from the subject and assayed in vitro.

The polynucleotides of the present invention are usually located on a vector. As well encompassed by the present invention is therefore a vector comprising at least one copy of the polynucleotides of the present invention.

The vector according to the invention is preferably an autonomously or self-replicating plasmid, a cosmid or a phage. A wide variety of host/vector combinations may be employed in expressing the polynucleotides of this invention. Useful vectors, for example, may consist of segments of chromosomal, non-chromosomal and/or synthetic nucleotide sequences. Suitable vectors include vectors with specific host range such as vectors specific for e. g. E. coli as well as vectors with broad host range such as vectors useful for gram-negative bacteria. A useful vector for expression in E. coli is e. g. pBAD/HisB (Invitrogen) which is preferred. Further useful plasmids are well known to the person skilled in the art and are described e.g. in "Cloning Vectors" (Eds. Pouwels P. H. et al. Elsevier, Amsterdam-New York-Oxford, 1985). A vector provided herewith which contains SEQ ID NO: 2 is pUC(OmpA).

The polypeptides or the antigenic fragments of the present invention can be produced in various prokaryotic or eukaryotic host cells which can be transformed with the respective polynucleotides. Thus, a further object of the present invention is a prokaryotic or eukaryotic host cell transformed with the polynucleotides and/or the vector of the present invention.

Transformation of appropriate host cells with a vector comprising a polynucleotide according to the invention is accomplished by well known methods that typically depend on the type of vector used. With regard to these methods, see for example, Maniatis et al. 1982, Molecular

Cloning, A laboratory Manual, Cold Spring Harbor Laboratory and commercially available methods.

A wide variety of prokaryotic host cells is useful in expressing the nucleotide sequences of this invention. These hosts may include strains of gram-negative cells such as *E. coli* and

- 5 *Pseudomonas*, or gram positive cells such as *Bacillus*. Useful eukaryotic host cells are e. g. insect cells which can be used together with a baculovirus expression system. Preferably, the host cell is a prokaryotic host cell, more preferably a gram-negative cell, most preferably an *E. coli* cell.

- 10 A further object of the present invention are the above mentioned primers for amplifying DNA encoding an OmpA polypeptide of *Rickettsia helevtica* selected from the group consisting of:
- a) a primer comprising the sequence SEQ ID NO: 33, an oligonucleotide complementary to SEQ ID NO: 33, an oligonucleotide consisting of 10- 50, preferably 15-30, nucleotides in length which hybridizes to SEQ ID NO: 33 or its complement under stringent conditions, an
- 15 oligonucleotide which is at least 60 %, preferably at least 70 %, more preferably at least 80 %, most preferably at least 90 % in particular at least 95 %, identical to the polynucleotide comprising the nucleotide sequences SEQ ID NO: 33;
- b) a primer comprising the sequence SEQ ID NO: 34, an oligonucleotide complementary to SEQ ID NO: 34, an oligonucleotide consisting of 10- 50 nucleotides, preferably 15-30, in
- 20 length which hybridizes to SEQ ID NO: 34 or its complement under stringent conditions, an oligonucleotide which is at least 60 %, preferably at least 70 %, more preferably at least 80 %, most preferably at least 90 % in particular at least 95 %, identical to the polynucleotide comprising the nucleotide sequences SEQ ID NO: 34.

- 25 Yet a further object of the present invention are primers and/or probes for amplifying a target DNA sequence of the OmpB gene of *Rickettsia helevtica* selected from the group consisting of:

- a) a primer comprising the sequence SEQ ID NO: 35, an oligonucleotide complementary to SEQ ID NO: 35, an oligonucleotide consisting of 10- 50 nucleotides, preferably 15-30, in
- 30 length which hybridizes to SEQ ID NO: 35 or its complement under stringent conditions, an oligonucleotide which is at least 60 %, preferably at least 70 %, more preferably at least 80 %, most preferably at least 90 % in particular at least 95 %, identical to the polynucleotide comprising the nucleotide sequences SEQ ID NO: 35;

most preferably at least 90 % in particular at least 95 %, identical to the polynucleotide comprising the nucleotide sequences SEQ ID NO: 35;

b) a primer comprising the sequence SEQ ID NO: 36, an oligonucleotide complementary to SEQ ID NO: 36, an oligonucleotide consisting of 10- 50, preferably 15-30, nucleotides in

5 length which hybridizes to SEQ ID NO: 36 or its complement under stringent conditions, an oligonucleotide which is at least 60 %, preferably at least 70 %, more preferably at least 80 %, most preferably at least 90 % in particular at least 95 %, identical to the polynucleotide comprising the nucleotide sequences SEQ ID NO: 36;

c) a probe comprising the sequence SEQ ID NO: 37, an oligonucleotide complementary to
10 SEQ ID NO: 37, an oligonucleotide consisting of 10- 60, preferably 15-40, nucleotides in length which hybridizes to SEQ ID NO: 37 or its complement under stringent conditions, an oligonucleotide which is at least 60 %, preferably at least 70 %, more preferably at least 80 %, most preferably at least 90 % in particular at least 95 %, identical to the polynucleotide comprising the nucleotide sequences SEQ ID NO: 37.

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As a basis to select primers and/or probes for amplifying a target DNA sequence of the OmpB gene of *Rickettsia helvetica*, database sequences such as GenBank or EMBL, fragments described in the literature or isolated fragments of the respective microorganism can be used.

The primers and/or probes for amplifying a target DNA sequence of the OmpB gene of
20 *Rickettsia helvetica* as defined above allow species-specific detection and/or monitoring of *R. helvetica*.

The present invention thus further comprises a method for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject, comprising assaying a biological fluid of the

25 subject with primers and/or probes for amplifying a target DNA sequence of the OmpB gene as defined above. Typical examples of assays which can utilize the primers and probes are polynucleotide amplification procedures which permit amplification of a target polynucleotide such as polymerase chain reaction (PCR), nested PCR or real time PCR, which are preferred.

30 Conditions which permit amplification of a target DNA sequence are well known to the person skilled in the art. In case a PCR method is used as amplification method e. g. a DNA polymerase such as Taq DNA polymerase (Perkin Elmer) or AmpliTaq (PE Applied

Biosystems), Hot GoldStar Taq polymerase (Eurogentec), AmpliTaq Gold polymerase (PE Applied Biosystems), SureStart Taq(Stratagene) or Platinum Taq (Gibco) can be used. The treatment to denature into a single-stranded DNA is usually a thermal treatment. PCR conditions used for amplification are known to the person skilled in the art.

5

The presence and amount of the amplified target DNA sequences can be detected by e. g. a method in which the size of the amplified target DNA sequences is confirmed by electrophoresis, by hybridizing the amplified target DNA sequences with a labelled probe having a sequence complementary to the sequence of the target amplified or by real-time PCR, wherein the amplified target DNA sequences are detected during amplification e. g. by measuring fluorescence during amplification using a variety of fluorescent dyes emitting at different wavelengths. Different fluorescent dyes and non-fluorescent quenchers depending on real-time PCR the system used can be coupled with the probe prior to amplification e. g. a fluorescent dye such as FAM (6-carboxyfluorescein), HEX(6-carboxy-2', 4,4',5', 7,7'-hexachlorofluorescein), TET (6-carboxy-4,7,2', 7'-tetrachloro-fluorescein), JOE (6-carboxy4', 5'-dichloro-2', 7'-dimethoxy fluorescein) or Texas-Red'can be coupled at the 5'-end of the probe and a fluorescent quencher such as TAMRA (6-carboxy-N, N, N', N-tetramethylrhodamine) or a non-fluorescent quencher such as DABCYL(4- (4-(dimethylamino) phenyl) azo) benzoic acid) can be coupled at the 3'-end of the probe. In case real-time PCR is used, usually the cycle threshold (Ct) of the amplified target DNA sequences is detected

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The present invention further comprises a diagnostic kit for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject, comprising the primers and/or probes for amplifying a target DNA sequence of the OmpB gene of *Rickettsia helevtica* as defined above.

Examples

Example 1

Collection of ticks

A total of 100 *Ixodes ricinus* ticks were collected using the cloth dragging method. We
5 collected females, males and nymphs in the region of Zuerich and Luzern in Switzerland.
Ticks were identified to species level and development stage and stored at 4°C in tubes with
high humidity.

DNA extraction

10 Prior to DNA isolation, ticks were disinfected in 70% ethanol, dried and mechanically
crushed. Lysis of the tick samples was carried out in the presence of 6.7 % saccharose solution,
0.2 % proteinase K, 20 mg/ml Lysozyme and 10 ng/ml RNase A for 16 h by 37°C. 0.5 M
EDTA and 20 % SDS was added and further incubated for 1 h at 37°C.
Extraction was performed twice with 80 % Phenol (1:1, v:v) and
15 Methylenechloride/Isoamylalcohol (24:1, v:v). DNA was precipitated with isopropanol. The
DNA-pellet was washed with 70 % ethanol and centrifuged at 16000 x g for 15 min. After
removal of the ethanol the pellet was dried at 50°C and dissolved in 25 - 50 µl distilled water.

Example 2

20 Nested PCR

DNA isolated according to example 1 from male and female ticks was used for PCR. A
specific 593-bp region of the *Rickettsia helvetica* 16S ribosomal RNA gene was amplified by
nested PCR. First-round PCR amplified a fragment of 736-bp of the 16S ribosomal RNA gene
of *Rickettsia* using primers 16SRf 5' ACGCTATCGGTATGCTTAACAC 3' and 16SRr 5'
25 GGACTACCAGGGTATCTAATCC 3'. First-round PCR was performed in 25-µl reaction
mixture containing 2 µl of isolated DNA, in Thermocycler (MWG, München, Germany) with
the following PCR profile: initial 10 min denaturation step at 95°C, followed by 40 cycles
consisting of denaturation at 95°C for 30s, primer annealing at 50°C for 30s and polymerase

extention at 72°C for 90s. Primers 16SRhv 5' GTGGGAATCTACCCATCAGTAT 3' and 16SRhr 5' CGTCAGTTGTAGCCCAGATGAT 3' amplified a specific 593 bp fragment of the PCR product generated in the first-round PCR. For the second-round PCR, 5 µl of the purified first-round reaction PCR was added to 20 µl reaction mixture. The thermal cycling
5 programm of the second-round PCR consisted of an initial 10 min denaturation step at 95°C, followed by 40 cycles consisting of denaturation at 95°C for 30s, primer annealing at 55°C for 30s and extention at 72°C for 60s. The Qiagen HotStarTaq Master Mix according to Qiagen protocols was used for PCR.

DNA electrophoresis was carried out in 1 % agarose gels containing ethidium bromide, and
10 DNA fragments were visualized under ultraviolet light. The sequence of the PCR product was confirmed by DNA sequencing. The sequence obtained from the PCR product is 100% identical to the 16S rRNA sequence of *R. helvetica* strain C9P9 (Genbank Accession No.: L36212).

15 593 nt partial 16S rDNA sequence of the amplified PCR product, identified as *R. helvetica*:
gtgggaatctacccatcagtatggaataacttttagaaataaaagctaataccatatattctctatggaggaaa
gattatcgctgatggatgagcccgctcagattaggtagttggtgaggtaacggctcaccaagccgacgatctgtagctggcttgagag
gatgatcagccacactgggactgagacacggcccagactcctacgggaggcagcagtgagggaatattggacaatggcgaaagcct
gatccagcaataccgagtgagtgatgaaggccttaggggtgtaaagctcttttagcaaggaagataatgacgttacttgcagaaaaagcc
20 ccggctaactccgtgccagcagccgcggaagacggagggggctagcggtgttcggaattactgggcgtaaagagtgcgtaggcggtt
tagtaagttggaagtgaagcccggggcttaacctcggaattgctttcaaaactactaatctagagtgtagtaggggatgatggaattccta
gtgtagaggtgaaattcttagatattaggaggaacaccggtggcgaaggcgatcatctgggctacaactgacg

Example 3

25 Identification of *Rickettsia helvetica* based on the PS 120 gene

A second specific PCR method was developed for detection of *R. helvetica* based on the PS 120 gene (Accession No: AF163009, Sekeyova et al., 2001, Int. J. Syst. Evol. Microbiol. 51 (PT 4), 1353-1360). Isolated DNA used in Example 2 from male and female ticks containing

Rickettsia helvetica was used for PCR. Specific primers PS120_for
TGATCCTGTTTTAGAGGCTCATTCAGAAG and PS120_rev
CTTTAGCTATATCTAAAGGATCACGAAAAGTACTT were used to amplify a PCR
product of 128 bp. The Primers were specific for R. helvetica. A primer annealing temperature
5 of 60°C was used for PCR. The resultant PCR product of 128 bp was sequenced using Big dye
terminator chemistry (Applied Biosystems) and DNA sequencer ABI PRISM 310.
The sequence obtained from the PCR product is 100% identical to the PS 120 gene sequence
of R. helvetica (Genbank Accession No.: AF163009). This sequence is unique for R.
helvetica.

10 128 nt partial PS 120 sequence of the amplified PCR product, identified as R. helvetica:
CTTTAGCTATATCTAAAGGATCACGAAAAGTACTTTTTATTCTCTCGACATAACTA
GAGATATCATTTAATACTAGAAAGTTTTTTTCGCCATTTGTTCTTCTGAATGAGC
CTCTAAAACAGGATCA

15 **Example 4**

Vector and cloning of DNA encoding OmpB of R. helvetica

Isolated DNA used in Example 2 from male and female ticks containing Rickettsia helvetica
was used for PCR. PCR amplification reactions were performed using the oligonucleotide
20 primer pair ompB_f (5' aatctagatgttggtgattc 3') and ompB R.h. NT_r (5' attatgcagcaaccgcgc
3'). The primers were designed with the OmpB sequence of R. helvetica (Genbank Accession
No. AF123725) without the signal sequence and without the autotransporter domain beta-
domain, which is described by Uchiyama, T.; 1999, Microbiol. Immunol., 43, 1061-1065. For
the PCR, the Triple Master PCR System (Eppendorf, 954140261) was used. Two microlitres
25 of the DNA preparation was amplified in a 50µL reaction mixture, containing 200nM of each
primer, 200µM dNTP mix (Roche), 5µL High Fidelity Buffer with Mg²⁺ and 0.75µL Triple
Master Polymerase Mix. The PCR was subjected to one initial denaturation step at 94°C for 2
min and then amplified in 35 cycles of 94°C for 20 sec, 54°C for 20 sec and 68°C for 3 min,

followed by a single 7-min extension step at 68°C. The resulting PCR product of 3919bp was purified using the QIAquick PCR purification kit (Qiagen) and directly ligated in the vector pCRT7/NT-TOPO (Invitrogen) by using the pCRT7 TOPO TA Expression Kit (Invitrogen). The ligation-mix was transformed in competent *E. coli* TOP 10F' cells and the transformants were selected and analysed for the integration of the ompB PCR product in the vector pCRT7/NT TOPO by sequencing. The sequencing reactions were carried out twice in both directions using 4µL of the resultant recombinant plasmid construct, named pCRT7/NT TOPO-ompB, 1µL of the PCR primer T7_f (5' taatacgactcactataggg 3'), T7_r (5' ctagttattgctcagcggtgg 3') respectively and 2µL BigDye terminator cycle sequencing mix (Applied Biosystems). The cycle sequencing was started by one initial denaturation step at 96°C for 2 min and then amplified in 28 cycles of 96°C for 10 sec, 50°C for 5 sec, 45°C for 5 sec, 40°C for 5 sec and 60°C for 3 min. The sequencing products were determined with the ABI Prism 310 Genetic Analyser (Applied Biosystems). Both partial ompB sequencing products showed a 100% identity with the ompB sequence of *R. helvetica* (Genbank Accession No. AF123725):

ompB, *R. helvetica*, 3'end, 245 nucleotides

aatctagatgttggtggtattcctgggatagtaacaggtgattctttaactatgtgtacaagcaggcggccttgactgctacggctaccg
gaggaaatgataacgggtggtaatgctacagtacttttcggttcgattaacatgttacaaaacgggtgttttcagttaacgggtgcagatatag
ctattggttctgtttccggtactgcaggtcaattattaacagttaattgcaggaaa

ompB, *R. helvetica*, 5'end, 328 nucleotides

aacgtagttactaatgatatcgaaatggtccgtttggaggtgcacctgggtgtaggtcagaacgttacaacatttgtaaatgcaactaatacc
gcagcatataataatcttcttttagctaaaaatagtgtgattctgctaactttgtcggaactattactaccgatacaagtcagccgtaactaa
tgcacaattagatatagctaaagatatccaagctcaacttggtaacagattaagtgcgcttagatattaggtactcctgaaactgctgaaat
gactgggcctgaagctggagcagtagccggctgcggttgctgcataat

Example 5

Vector and cloning of DNA encoding the OmpA antigen

Isolated DNA used in Example 2 from male and female ticks containing *Rickettsia helvetica* was used for PCR. DNA encoding the OmpA antigen with its signal sequence was amplified by PCR using forward Primer actgtcgagctcgatggcgaatatttctccaaaaatta which created *SacI* recognition site (underlined) and reverse Primer tgaccggaattcattaaaaattaacacgaactttcacac which created *EcoRI* recognition site (underlined). Both primers were designed based on alignments using the program ClustalW of homologous OmpA sequences of *Rickettsia* species available in the NCBI Genbank. PCR was performed in a Primus-HT thermocycler (MWG BIOTECH) with 1 µg, or less, of template DNA in a total reaction volume of 50 µl with 2.5 U TripleMaster Polymerase Mix (Triple Master PCR System Eppendorf, 954140261), 200 µM dNTP, 200 nM of forward and reverse primer, respectively. Cycling parameters was 94°C for 2 min followed by 35 cycles of 94°C for 20 sec, 48°C for 20 sec and 68°C for 6 min followed by a final 7 min extension step at 68°C. The PCR product after purification using QIAquick PCR purification kit (Qiagen) was digested with *SacI* and *EcoRI* restriction enzymes and ligated after a second purification step with *SacI* and *EcoRI* digested purified expression vector pBAD/HisB (Invitrogen). The ligation mix was used to transform *E. coli* TOP 10F'. Transformants were selected and analysed for the integration of the OmpA-PCR-product in the vector pUC18. The resultant recombinant plasmid was named pUC(OmpA). pUC(OmpA) was partially sequenced using M13rev 5' (CAG GAA ACA GCT ATG AC) and M13vor (TGT AAA ACG ACG GCC AG) primers using Big dye terminator chemistry (Applied Biosystems) and DNA sequencer ABI PRISM 310. The full-length sequence was determined by Microsynth (Balgach, Switzerland) using the DNA sequencing service. The OmpA protein sequence of *Rickettsia helvetica* obtained has the following identity with ompA sequences of other *Rickettsia*:

66% identity with partial ompA sequence of *R. slovaca* (ompA sequence of *R. slovaca* 13-B, comprising 1060 amino acids, Genbank Accession No.: AAC35190);

63% identity with complete ompA-sequence of *R. australis* strain PHS (Genbank Accession No.: AAD39531);

51% identity with with complete ompA-sequence of *R. sibirica* strain 246 (Genbank Accession No.: ZP_00142612);

50% identity with complete ompA-sequence of *R. conorii* strain Malish (Genbank Accession No.: AAL03811);

5 49% identity with complete ompA-sequence of *R. africae* strain ESF 2500-1 (Genbank Accession No.: AAC35172);

48% identity with complete ompA-sequence of *R. rickettsii* (Genbank Accession No.: ZP_00154221).

10 Based on the OmpA protein sequence, which is composed of 2015 amino acids, the available computer programs: Antigen design tool (http://www.genscript.com/cgi-bin/tools/antigenic_prediction.pl, GenScript Corporation) and Predicting antigenic peptides (<http://bio.dfci.harvard.edu/Tools/antigenic.pl>, MIF: Molecular Immunology Foundation) were used to determine the antigenic fragments from OmpA shown in Table 1.

15

Table 1

position OmpA protein	SEQ ID NO:	antigenic fragment	position DNA sequence	SEQ ID NO:	corresponding DNA sequence
406 - 420	18	TGGSALKQLTFNN VV	1216 - 1260	3	ACCGGTGGTAGTGCA CTAAAGCAATTAACA TTTAATAACGTAGTA
507 - 521	19	TGGSALKQLTFNN VV	1519 - 1563	4	ACCGGTGGTAGTGCA CTAAAGCAATTAACA TTTAATAACGTAGTA
67 - 81	20	YTDDDFTVDADE AVN	199 - 243	5	TATACAGATGACGATT TTACGGTTGATGCTGA TGAAGCAGTAAAT
87 - 100	21	VNNKVPALFTVN KN	259 - 300	6	GTAAACAATAAAGTA CCTGCTTTATTTACGG TAAATAAAAAT
49 - 63	22	STGVGLNPAVKF AGG	145 - 189	7	AGTACAGGTGTGGGG TTAAATCCTGCTGTAA AATTTGCAGGCGGT
194 - 218*	23	TTHKLASITVDGG VGVGAIslaATN	580 - 654	8	ACAACCCATAAGTTA GCTTCAATAACTGTTG ATGGTGGTGTAGGCG TAGGTGCTATTTCGCT

					TGCAGCAACAAAT
393 - 407	24	TVIVTAGDIANAG TG	391 - 1221	9	ACGGTTATTGTAACGG CAGGTGATATAGCAA ATGCCGGTACCGGT
494 - 518	25	TVIVTAGDIANAG TG	1480 - 1554	10	ACGGTTATTGTAACGG CAGGTGATATAGCAA ATGCCGGTACCGGT
657 - 675*	26	AAGKLIAGGLVT GTAVLTT	1969 - 2025	11	GCTGCCGGTAAGTTA ATTGCCGGTGGGTTAG TAACTGGTACAGCAG TATTA ACTACA
758 - 776*	27	AAGKLIAGGLVT GVAVFNA	2272 - 2328	12	GCTGCTGGTAAATTA TCGCCGGTGGGTTAGT TACAGGTGTAGCAGT GTTTAATGCA
173 - 187	28	ANDDEGV LKVST DTT	517 - 561	13	GCAAATGACGATGAG GGTGTATTA AAAAGTTA GTACAGATACCACT
295 - 309	29	VIANKGLKLVTFN NG	883 - 927	14	GTTATAGCTAATAAAA GGGCTAAAATTAGTA ACTTTTAATAACGGT
242 - 257	30	AKFVAGGLVTGT AVFN	724 - 771	15	GCAAAATTTGTAGCA GGCGGTTTAGTCACG GGTACTGCAGTATTTA AT
597 - 611	31	VIVTAGDIANAGA NG	1789 - 1833	16	GTTATAGTAACAGCA GGAGATATAGCAAAT GCCGGTGCAAATGGT
697 - 711	32	VIVTAGDIANAGT GG	2089 - 2133	17	GTTATAGTAACAGCA GGAGATATAGCAAAT GCTGGTACCGGTGGT

Example 6

ELISA Assay to detect *Rickettsia helvetica* infections

Peptides were synthesized by the Thermo Electron Corporation, Ulm, Germany. The amino
5 terminus of the peptides was biotin labeled and a spacer was introduced between each peptide
and biotin.

For the ELISA, microtiter plate wells coated with streptavidin HBC (Reacti-Bind™
Streptavidin High Binding Capacity [HBC] Coated Plates, Pierce) were washed with 3 x

200µL of wash buffer (100mM sodium phosphate, 150mM NaCl, pH 7.2, 0.05% Tween-20) followed by the addition of 100µL of the biotinylated peptide (1-10ng/µL)/well. Plates were incubated for 1 hour at room temperature by shaking with 4500rpm. After the incubation time each well was washed with 3 x 200µL of wash buffer. After the wash, 100µL of human blood
5 serum (1:100 diluted in blocking buffer [100mM sodium phosphate, 150mM NaCl, pH 7.2, 0.05% Tween-20, 0.1% BSA]) was added to each well and the microwell plate was incubated for 30min at room temperature by shaking with 4500rpm. After this second incubation step each well was washed with 3 x 200µL of wash buffer. After the wash, 100µL of goat anti human horse radish peroxidase-labeled secondary antibody specific for IgG or IgM (IgG
10 1:3000 diluted in blocking buffer and IgM 1:20'000 diluted in blocking buffer, respectively) was added to each well and the microwell plate was incubated for 30 minutes at room temperature by shaking with 4500rpm. After the incubation step with the diluted serum sample, each well was washed with 3 x 200µL of wash buffer. The microwell plate was developed by adding 100µL of TMB-ELISA substrate solution (1-Step™ Ultra TMB-
15 ELISA, Pierce) to each well and each plate was incubated for 15 minutes at room temperature. The reaction was stopped by adding 100 µL of 2M sulfuric acid to each well and the microwell plate was incubated over night at room temperature. Optical density of wells was read at 450nm for IgG as shown in table 3.

In addition, microtiter plates were coated with the peptide/well in carbonate buffer (90 mM
20 NaHCO₃ and 60mM Na₂CO₃, pH 9.6), covered and incubated at 4°C for 16-18 h. Blocking was performed 2 h with 300 µL milk powder (10%) in PBS buffer containing 0.05% tween 20 per well. Washing steps, incubation with diluted human serum samples were performed as described above. Serum samples were diluted 1:10 to 1:100. Optical density of wells was read immediately at 450 nm for IgG as shown in table 2. Optical density of wells was read
25 immediately at 450 nm for IgM as shown in table 4.

For the developed ELISA, each microtiter plate contained human serum controls ranging in reactivity from non-reactive (negative control) to highly reactive (positive control).

- Human sera used are: 411: negative control (no infection with *Rickettsia*); F1: Patient, 1127: Patient, 1126: Patient, 4290410 (26): Patient with *R. conorii* infection, 2339140: Erythema migrans patient with prevalence of IgG and IgM antibodies against *R. conorii* as found by Indirect fluorescence antibody assay (IFA) for the detection of IgG and IgM antibodies against
- 5 *R. conori*, 3147357: Erythema migrans patient with prevalence of IgG and IgM antibodies against *R. conorii* as found by Indirect fluorescence antibody assay (IFA) for the detection of IgG and IgM antibodies against *R. conori*, 4234352: negative control (no infection with *Rickettsia*), 4255924: Patient, presumably infected with *R. conorii* and/or *R. africae*.
- Peptides specific for *R. helevtica* used are: Ra194 (TTHKLASITVDGGVGVGAISLAATN),
- 10 Ra87 (VNNKVPALFTVNKN), Ra657 (AAGKLIAGGLVTGTAVLTT), Ra406/507(TGGSALKQLTFNNVV), Ra49 (STGVGLNPAVKFAGG). Peptides Rsp971, Rsp954, RspompB, RspompA were used which react with antibodies in serum samples directed against *Rickettsia* species.

15 **Table 2**
OD 450nm, peptide coated plate,
IgG

Human serum	Peptides								
	Ra194	Ra87	Ra657	Ra406/507	Rsp971	Rsp954	RspompB	RspompA	BSA
411	0,049	0,049	0,052	0,048	0,051	0,045	0,045	0,045	0,047
F1	0,054	0,453	0,047	0,274	0,083	0,193	0,184	0,085	0,043
1127	0,061	0,046	0,044	0,045	0,057	0,061	0,047	0,064	0,043
1126	0,631	0,412	-	-	0,081	0,060	0,083	0,217	0,044
4290410 (26)	0,052	0,388	0,131	0,071	0,227	0,061	0,061	0,054	0,046
2339140	0,041	0,041	0,137	0,105	0,102	0,079	0,106	0,087	0,047
4234352	0,049	0,042	0,042	0,044	0,044	0,041	0,048	0,053	0,048
4255924	0,099	0,044	0,041	0,047	0,048	0,047	0,042	0,281	0,045

all peptides were directly coated with carbonat buffer, cut off:
0,1

Table 3

OD 450nm, streptavidine coated plate (pierce), IgG

Human serum	Peptides								
	Ra194	Ra87	Ra657	Ra406/507	Rsp971	Rsp954	RspompB	RspompA	BSA
1127	0,073	0,164	0,115	0,231	0,086	0,148	0,066	0,182	0,050
3147357	0,260	0,573	0,216	0,784	0,202	1,023	0,141	0,575	0,145
2339140	0,055	0,388	0,660	0,373	0,136	0,626	0,121	0,242	0,133

cut off:
0.3

Table 4**OD 450nm, peptide coated plate, IgM**

Human serum	Peptides									
	Ra194	Ra87	Ra657	Ra406/507	Ra49	Rsp971	Rsp954	RspompB	RspompA	BSA
411	0,049	0,066	0,159	0,103	0,073	0,078	0,073	0,071	0,175	0,141
2339140	0,176	0,173	0,241	0,300	0,214	0,191	0,180	0,200	0,304	0,146
3147357	0,170	0,114	0,122	0,117	0,100	0,118	0,092	0,099	0,143	0,100

all peptides were directly coated with carbonat buffer
cut off:
0.2

Example 7**Real-time PCR for detection of *Rickettsia helvetica* in blood and ticks**

- 5 The TaqMan probe and the primer sequences for detection of *Rickettsia helvetica* based on the ompB gene were designed with the software program Primer Express (Applied Biosystems). A 162 bp fragment of the ompB gene of *R. helvetica* (AF123725, region 2983 to 3144) is amplified with the forward primer Rh_ompB_F: GATTTCGACGGTAAAATTACC and reverse primer Rh_ompB_R: GCT ACCGATATTACCTACAG. The TaqMan probe
- 10 Rh_ompB_P: ACTCTACTGCTACAA GTATGGTTGCTACAG was designed. The fluorescent dyes at the 5' end was FAM and at the 3' end TAMRA, respectively.

The absence of homology of the used primers and probe with unrelated sequences was determined with BLAST search, a program for sequence analysis of the NCBI.

Standard curve was generated with serial dilutions from 10^8 to 10^1 copies of the plasmid which contains the vector (pCR2.1, Invitrogen) with the 162 bp fragment of OmpB. Slope,

5 Y-intercept and correlation coefficients of the standard curve are: -3.634, 40.129 and 0.9937, respectively.

DNA from whole blood was isolated according to the instructions of Machery-Nagel, CH-Oensingen, Genomic DNA from Blood (April 2005/Rev. 04).

The 30- μ l (final volume) real-time PCR mixture contained 15 μ l Master Mix (Applied Biosystems) 900 nM of each primer, 250 nM of the TaqMan probe and 5-10 μ l of the template
10 DNA or diluted plasmid standard. Real-time PCR was performed using ABI 7000 (Applied Biosystems). The amplification program consisted of initial heating at 95°C for 15 min, followed by 40 cycles of heating at 95°C for 20s, primer annealing and polymerization at 60°C for 1 min. The copy numbers of detected *Rickettsia helvetica* based on the developed
15 ompB real-time PCR assay in ticks is in the range of 10 to 10'000 per tick, corresponding to CT-values of 37.3 and 27, respectively.

Claims

1. A polynucleotide encoding an OmpA polypeptide of *Rickettsia helvetica* selected from the group consisting of:

5 a) polynucleotides encoding the OmpA polypeptide of *Rickettsia helvetica* comprising the amino acid sequence as shown in SEQ ID NO: 1;

b) polynucleotides comprising the nucleotide sequence as shown in SEQ ID NO: 2;

c) polynucleotides the complementary strand of which hybridizes under stringent conditions to a polynucleotide as defined in (a) or (b);

10 d) polynucleotides which are at least 80 % identical to a polynucleotide as defined in (a) or (b).

2. A polynucleotide encoding an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* selected from the group consisting of:

15 a) polynucleotides encoding an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* comprising at least 5 consecutive amino acids located from amino acid 1 to amino acid 800 of the amino acid sequence as shown in SEQ ID NO: 1;

b) polynucleotides comprising a nucleotide sequence selected from SEQ ID NOS: 3-17;

c) polynucleotides which are at least 60 % identical to a polynucleotide comprising a nucleotide sequence selected from SEQ ID NOS: 3-17.

20 d) polynucleotides which are at least 90 % identical to a polynucleotide comprising a nucleotide sequence selected from SEQ ID NOS: 3-17.

3. The polynucleotide of claim 2 as defined in a) encoding an antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* comprising at least 5 consecutive amino acids located from about amino acid 49 to about amino acid 100 and/or from about amino acid 173 to about amino acid 309 and/or from about amino acid 393 to about amino acid 521 and/or from about amino acid 597 to about amino acid 776 of the amino acid sequence as shown in SEQ ID No 1.

4. The polynucleotide of claim 2 as defined in c), whereas the nucleotide sequence is selected from SEQ ID NOS: 3, 4, 6, 8, 10, 11, 12, 14, and 17.

5. The polynucleotide of claim 2 as defined in d), whereas the nucleotide sequence is selected from SEQ ID NOS: 5, 7, 9, 13, 15, and 16.

6. An OmpA polypeptide of *Rickettsia helvetica* having the amino acid sequence encoded by the polynucleotide of claim 1.

7. An OmpA polypeptide of *Rickettsia helvetica* selected from the group consisting of
a) polypeptides comprising the amino acid sequence as shown in SEQ ID NO: 1;
b) polypeptides comprising an amino acid sequence which is at least 80 % identical in amino acid sequence to the amino acid sequence as defined in (a).

8. An antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* having the amino acid sequence encoded by the polynucleotide of claims 2-5.

9. An antigenic fragment of an OmpA polypeptide of *Rickettsia helvetica* selected from the group consisting of

a) an antigenic fragment comprising at least 5 consecutive amino acids located from amino acid 1 to amino acid 800 of the amino acid sequence as shown in SEQ ID NO: 1;

b) an antigenic fragment comprising an amino acid sequence selected from SEQ ID NOS: 18-32;

c) an antigenic fragment comprising an amino acid sequence which is at least 60 % identical to an amino acid sequence selected from SEQ ID NOS: 18-32;

d) an antigenic fragment comprising an amino acid sequence which is at least 80 % identical to an amino acid sequence selected from SEQ ID NOS: 18-32;

e) an antigenic fragment consisting of at least 5 consecutive amino acids of an amino acid sequence selected from SEQ ID NOS: 18-32.

10. The antigenic fragment of claim 9 as defined in a) comprising at least 5 consecutive amino acids located from about amino acid 49 to about amino acid 100 and/or from

about amino acid 173 to about amino acid 309 and/or from about amino acid 393 to about amino acid 521 and/or from about amino acid 597 to about amino acid 776 of the amino acid sequence as shown in SEQ ID No 1.

- 5 11. The antigenic fragment of claim 10 as defined in c), whereas the amino acid sequence is selected from SEQ ID NOS: 18-27 and 30-32.
12. The antigenic fragment of claim 10 as defined in d), whereas the amino acid sequence is selected from SEQ ID NOS: 28, 29.
- 10 13. The antigenic fragment of claim 10 as defined in b), c), d) and e), whereas the amino acid sequence is selected from SEQ ID NOS: 18, 19, 21, 23, and 26.
14. An antibody against a polynucleotide of any one of claims 1-5, an OmpA polypeptide of
15 claims 6 or 7 or an antigenic fragment of an OmpA polypeptide of any one of claims 8-13, for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject.
15. A method for detecting and/or monitoring the presence of *Rickettsia helvetica* in a
20 subject, comprising assaying a biological fluid of the subject with a compound selected from
- a) a polynucleotide of claim 1;
b) a polynucleotide of claims 2-5;
c) an OmpA polypeptide of claims 6 or 7;
d) an antigenic fragment of an OmpA polypeptide of any one of claims 8-13;
25 e) an antibody of claim 14.
16. The method of claim 15, wherein the method is a serological assay selected from enzyme-linked immunosorbent assays (ELISA), immunofluorescence assays (IFA), Western immunoblot assays and cross-absorption assays and, wherein the biological
30 fluid of the subject is assayed with a compound selected from
- c) an OmpA polypeptide of claims 6 or 7;
d) an antigenic fragment of an OmpA polypeptide of any one of claims 8-13;

e) an antibody of claim 14.

17. The method of claim 16, wherein the serological assay is an ELISA and the compound used is an antigenic fragment of an OmpA polypeptide of any one of claims 8-13.

5

18. A diagnostic kit for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject, comprising a compound selected from

a) a polynucleotide of claim 1;

b) a polynucleotide of claims 2-5;

10

c) an OmpA polypeptide of claims 6 or 7;

d) an antigenic fragment of an OmpA polypeptide of any one of claims 8-13;

e) an antibody of claim 14;

optionally with reagents and/or instructions for use.

15

19. The diagnostic kit of claim 18, wherein the compound is selected from an antigenic fragment of an OmpA polypeptide of any one of claims 8-13.

20. A vector comprising at least one copy of the polynucleotide of any one of claims 1-5.

20

21. The vector of claim 20 comprising the nucleotide sequence as shown in SEQ ID NO: 2.

22. A prokaryotic host cell transformed with the polynucleotide of any one of claims 1-5 and/or the vector of claims 20 or 21.

25

23. Primers for amplifying DNA encoding an OmpA polypeptide of *Rickettsia helvetica* selected from the group consisting of:

a) a primer comprising the sequence SEQ ID NO: 33, an oligonucleotide complementary to SEQ ID NO: 33, an oligonucleotide consisting of 10-50 nucleotides in length which hybridizes to SEQ ID NO: 33 or its complement under stringent conditions, an oligonucleotide which is at least 60 % identical to the polynucleotide comprising the nucleotide sequences SEQ ID NO: 33;

30

b) a primer comprising the sequence SEQ ID NO: 34, an oligonucleotide complementary

to SEQ ID NO: 34, an oligonucleotide consisting of 10-50 nucleotides in length which hybridizes to SEQ ID NO: 34 or its complement under stringent conditions, an oligonucleotide which is at least 60 % identical to the polynucleotide comprising the nucleotide sequences SEQ ID NO: 34.

5

24. Primers and/or probes for amplifying a target DNA sequence of the OmpB gene of *Rickettsia helvetica* selected from the group consisting of :

a) a primer comprising the sequence SEQ ID NO: 35, an oligonucleotide complementary to SEQ ID NO: 35, an oligonucleotide consisting of 10-50 nucleotides in length which hybridizes to SEQ ID NO: 35 or its complement under stringent conditions, an oligonucleotide which is at least 60 % identical to the polynucleotide comprising the nucleotide sequences SEQ ID NO: 35;

10

b) a primer comprising the sequence SEQ ID NO: 36, an oligonucleotide complementary to SEQ ID NO: 36, an oligonucleotide consisting of 10- 50 nucleotides in length which hybridizes to SEQ ID NO: 36 or its complement under stringent conditions, an oligonucleotide which is at least 60 % identical to the polynucleotide comprising the nucleotide sequences SEQ ID NO: 36;

15

c) a probe comprising the sequence SEQ ID NO: 37, an oligonucleotide complementary to SEQ ID NO: 37, an oligonucleotide consisting of 10- 60 nucleotides in length which hybridizes to SEQ ID NO: 37 or its complement under stringent conditions, an oligonucleotide which is at least 60 % identical to the polynucleotide comprising the nucleotide sequences SEQ ID NO: 37.

20

25. A method for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject, comprising assaying a biological fluid of the subject with the primers and/or probes of claim 24.

25

26. The method of claim 25, wherein the biological fluid of the subject is assayed with an amplification method selected from polymerase chain reaction (PCR), nested PCR or real time PCR.

30

27. A diagnostic kit for detecting and/or monitoring the presence of *Rickettsia helvetica* in a subject, comprising the primers and/or probes of claim 24.