TARGET GENES FOR STRAIN-SPECIFIC DIAGNOSTIC OF EHRLICHIA RUMINANTIUM AND USE THEREOF

The invention provides a combination of target genes that are useful as genetic markers for the strain-specific detection of Ehrlichia ruminantium. The invention also provides diagnostic methods using said combination of markers.
TARGET GENES FOR STRAIN-SPECIFIC DIAGNOSTIC OF *EHRlichia ruminantium* AND USE THEREOF

*Rickettsiae* are intracellular pathogenic bacteria responsible for various diseases on Humans and animals. *Rickettsiae* are transmitted by arthropods, most frequently ticks, lice and mites, and cause major illnesses such as epidemic typhus or Rocky Mountain spotted fever. The genus *Ehrlichia* comprises other species pathogenic for humans and mammals such as *E. chaffeensis*, responsible for Human monocytic ehrlichiosis, *E. canis*, the causing agent of canine monocytic ehrlichiosis.

Another species, *Ehrlichia ruminantium*, formerly known as *Cowdria ruminantium*, is the causing agent of heartwater or cowdriosis, an economically important disease of domestic ruminants. Heartwater can cause up to 80 % mortality in susceptible animals. *E. ruminantium* is transmitted by *Amblyomma* ticks and is present in Sub-Saharan Africa and surrounding islands, including Madagascar. Heartwater is also present in several Caribbean islands and is threatening the American mainland.

Vaccination against heartwater has long been based on “infection and treatment”. Naïve animals are inoculated with blood containing virulent organisms, a procedure which carries a high risk of uncontrolled clinical reactions and the inadvertent spread of undesirable parasites and viruses. A first generation cowdriosis inactivated vaccine based on cell-cultured derived elementary bodies was developed. Although representing a considerable improvement and the first heartwater vaccine acceptable for widespread use, the level of protection conferred is still not fully satisfactory. Indeed, all animals develop a clinical reaction at challenge despite vaccination. Furthermore, livestock also faces challenge by genetically and antigenically diverse strains.

Diversity of *E. ruminantium* is a key problem which has been recognized for a long time, but insufficient information is available for optimum vaccine formulation and specific diagnostic. Serological diagnostic tests of heartwater using crude antigens from whole bacteria detect false positive reactions due to common antigenic determinants.

The diversity of *E. ruminantium* was demonstrated at the antigenic level by cross-immunisation studies. Variable antigens were identified by ELISA and immunoblot using cross-absorbed immune sera.

Genetic diversity was later demonstrated when sequencing the Map 1 gene which showed a high degree of sequence heterogeneity concentrated in three hypervariable regions. Genomic polymorphism was also detected using RAPD and RFLP markers. This DNA polymorphism was shown to correlate with antigenic polymorphism.

ELISA-based and serological diagnostics have been developed using the Map 1 and the GroEL (WO 9914233) antigens. Other peptides for serological diagnostic have been described (US 2002004051, US 20020132789, WO 02/066652). Although they have dramatically improved specificity, they still display cross reaction with *E. canis* and *E.
*chaffeensis*. The map1 gene initially considered as a good marker for geographic diversity, was recently shown not to be geographically constrained. Furthermore, the life span of anti-Map 1 antibodies is rather short.

PCR-based diagnostic methods represent methods of choice for the sensitive and specific detection of *Ehrlichia* in clinically reactive or asymptomatic carrier ruminants, as well as in vectors. However, in the field, hosts and vectors can be co-infested by several parasites and the diversity of pathogen species is further complicated by the existence of extensive intra-species diversity. Thus, it is important to provide means and diagnostic tools allowing not only to identify *E. ruminantium* but also to differentiate between different strains.

Sequences allowing differential diagnostic of *E. ruminantium* strain Gardel and *E. ruminantium* strain Welgevonden have been previously described by the inventors. They have shown, through complete genome sequencing and comparative genomic analysis that several genes were only found in either strain Gardel or strain Welgevonden, without counterpart in the other strain, and that several other genes, while being present in both strains differed between them by one or several mutations, such as large insertions and/or deletions that result in a frameshift and/or in a truncated version of the original gene. These genes were therefore primary targets to develop specific, multitarget diagnostic methods to differentiate between these two strains (WO 2006/045338; Frutos et al., Journal of Bacteriology.188:2533-2542, 2006).

The inventors have now found that the use of a particular combination of some of the target genes described in WO 2006/045338 allowed not only to discriminate between strains Gardel and Welgevonden, but also in a more general way, to detect specifically *E. ruminantium* and to discriminate between a broad range of strains of *E. ruminantium* other than Gardel and Welgevonden including strains for which no genomic sequence data are available.

An object of the invention is thus the use of the following set of genes:

- Erum1, defined by the sequence SEQ ID NO: 6
- Erum2, defined by the sequence SEQ ID NO: 3
- Erum3, defined by the sequence SEQ ID NO: 1
- Erum4, defined by the sequence SEQ ID NO: 4
- Erum5, defined by the sequence SEQ ID NO: 2
- Erum6, defined by the sequence SEQ ID NO: 5
- Erum7, defined by the sequence SEQ ID NO: 13
- Erum8, defined by the sequence SEQ ID NO: 15
- Erum9, defined by the sequence SEQ ID NO: 14
- Erum10, defined by the sequence SEQ ID NO: 8,

as targets for the strain-specific detection of *Ehrlichia ruminantium*. 
The reference sequences used herein to define the target genes Erum 1-5 and Erum 7-9 are those identified in the Gardel strain; the reference sequences used herein to define the target genes Erum6 and Erum10 are those identified in the Welgevonden strain.

However, it is to be understood that each of these genes actually exists under different allelic forms, depending on the strain of *Ehrlichia ruminantium*. The allelic forms that will be considered herein, having in view strain-specific detection, are in particular those resulting from large insertions and/or deletions that lead to a frameshift or to a truncated version of the original gene.

The invention thus provides a method for the strain-specific detection of *Ehrlichia ruminantium* wherein said method comprises determining, for each of the genes Erum 1 to Erum10 defined above, whether said gene is present in the bacteria to be tested, and under which allelic form.

Advantageously, the method of the invention is carried out by performing PCR amplification of all the target genes Erum 1 to Erum10, and checking, for each of these genes, the presence of one or more amplification product(s), and the size of said amplification product(s).

Within the target genes Erum 1 to Erum10, preferred target regions are as follows:

For Erum 1, the target region can consist of the whole sequence SEQ ID NO: 6, or of a portion thereof; in particular the target region can be defined within the portion spanning from nucleotide 1 to nucleotide 173 of SEQ ID NO: 6.

For Erum 2, the target region can consist of the whole sequence SEQ ID NO: 3, or of a portion thereof; in particular the target region can be defined within the portion spanning from nucleotide 1 to nucleotide 218 of SEQ ID NO: 3.

For Erum 3, the target region can consist of the whole sequence SEQ ID NO: 1, or of a portion thereof; in particular the target region can be defined within the portion spanning from nucleotide 1 to nucleotide 509 of SEQ ID NO: 1.

For Erum 4, the target region can consist of the whole sequence SEQ ID NO: 4, or of a portion thereof; in particular the target region can be defined within the portion spanning from nucleotide 56 to nucleotide 698 of SEQ ID NO: 4.

For Erum 5, the target region can consist of the whole sequence SEQ ID NO: 2, or of a portion thereof; in particular the target region can be defined within the portion spanning from nucleotide 1 to nucleotide 239 of SEQ ID NO: 2.

For Erum 6, the target region can consist of the whole sequence SEQ ID NO: 5, or of a portion thereof; in particular the target region can be defined within the portion spanning from nucleotide 3 to nucleotide 130 of SEQ ID NO: 5.

For Erum 7, a preferred target region is located within the portion spanning from nucleotide 1 to nucleotide 1981 of SEQ ID NO: 13; another preferred target region is
located within the portion spanning from nucleotide 2378 to nucleotide 3252 of SEQ ID NO: 13.

For Erum 8, a preferred target region is located within the portion spanning from nucleotide 1 to nucleotide 926 of SEQ ID NO: 15; another preferred target region is located within the portion spanning from nucleotide 1816 to nucleotide 3570 of SEQ ID NO: 15.

For Erum 9, a preferred target region is located within the portion spanning from nucleotide 1 to nucleotide 1307 of SEQ ID NO: 14; another preferred target region is located within the portion spanning from nucleotide 151 to nucleotide 1836 of SEQ ID NO: 14.

For Erum 10, a preferred target region is located within the portion spanning from nucleotide 1 to nucleotide 598 of SEQ ID NO: 8; another preferred target region is located within the portion spanning from nucleotide 792 to nucleotide 3522 of SEQ ID NO: 8; still another target region is located within the portion spanning from nucleotide 599 to nucleotide 791 of SEQ ID NO: 8.

Various techniques for detection of target nucleic acid sequences based on PCR amplification are available in the art.

These methods include in particular combined PCR analysis, i.e. simultaneous gel visualization of ten individual PCR reactions, each one targeting only one of the genes Erum1 to Erum10 defined above. The ten target genes can also be analysed by multiplex PCR, by a single PCR reaction involving simultaneous amplification of all the genes using a mixture of primers and visualization of the pattern on electrophoresis gel, or by a combination of multiplex PCR reactions, each one concerning a subset of the target genes listed above.

Non-limitative examples of PCR primers allowing to carry out the method of the invention are given in Table 2 below. Other suitable PCR primers can easily be designed by one of skill in the art, on the basis of the information provided by the present invention. By way of non-limitative example of oligonucleotide design software suitable for obtaining PCR primers of the invention, one can mention the software Vector NTI Advance 9.0 (Invitrogen).

The invention also comprises diagnostic kits for discriminating between strains of *E. ruminantium* wherein said kits comprise PCR primers for all the target genes Erum 1 to Erum10.

The method of the invention is useful in particular to discriminate between strains of *E. ruminantium* other than strain Gardel and strain Welgevonden. It is also useful to discriminate between strain Gardel and strains of *E. ruminantium* other than strain Welgevonden, or conversely, between strain Welgevonden and strains of *E. ruminantium*
other than strain Gardel. Furthermore, it also allows for discriminating between a virulent strain of *E. ruminantium* and its attenuated counterpart.

The method of the invention can be performed either on whole bacteria previously lysed, or on nucleic acid (genomic DNA, cDNA or mRNA) isolated from said bacteria. It is suitable for use at various stages of the life cycle of *E. ruminantium*, more specifically but not limited to the domestic-ruminants infectious stage, vector-interaction stage or reservoir animals-interaction stage. Preferred utilisations of the method of the invention include the detection of *Ehrlichia ruminantium* in a given territory, the strain specific identification of *Ehrlichia ruminantium* in a given territory, the discrimination between strains of *Ehrlichia ruminantium* in a given territory or between different geographical regions, the analysis of strain movements within a region or between geographically distinct regions, the differential presence of strains of *Ehrlichia ruminantium* according to vector species and/or populations or the early detection and risk assessment in regions where potential vectors are present but where the disease has not been recorded yet.

Specifically exemplified herein is the identification of *E. ruminantium* strains based on the specific amplification patterns of the ten target genes defined above,

**EXAMPLE 1. GENERAL FEATURES AND SEQUENCE REFERENCE**

For each strain, purified DNA was broken by sonication to generate fragments of differing sizes. After filling up the ends with Klenow polymerase, DNA fragments ranging from 0.5 kb to 4 kb were separated in a 0.8% agarose gel and collected after gelase (Epicentre) digestion of a cut agarose band. Blunt-end DNA fragments were inserted into pBluescript II KS (Stratagene) digested with EcoRV and dephosphorylated. Ligation was performed with the Fast-Link DNA Ligation kit (Epicentre) and competent DH10B *E. coli* were transformed prior to colony isolation on LB-agar+ Ampicillin + Xgal +IPTG. About 15000 clones were isolated for each strain of *E. ruminatium*. Plasmidic DNA from recombinant *E. coli* strains was extracted according to the alkaline lysis method and inserts were sequenced on both strands using universal forward and reverse M13 primers and the ET DYEnamic terminator kit (Amersham). Sequences were obtained with ABI 373 et ABI 377 automated sequencers (Applied Biosystems). Data were analysed and contigs were assembled using Phred-Phrap and Consed software packages (http://www.genome.washington.edu). Gaps were filled in through primer-directed sequencing using custom made primers. A total of about 20000 raw sequence runs were generated and analysed for each *E. ruminantium* strain to generate a full length consensus sequence with a coverage of 6x to 7x.

*E. ruminantium* strain Gardel and *E. ruminantium* strain Welgevonden are virulent pathogenic strains causing heartwater in Guadeloupe Island (French West Indies) and South Africa, respectively. The genome of *E. ruminantium* strains Gardel and Welgevonden is arranged as a circular chromosome of 1499920 bp and 1512977 bp, respectively. The
respective G+C contents for the strains Gardel and Welgevonden is 27.51 % and 27.48 %.

The genome of *E. ruminantium* strain Gardel comprises 948 coding sequences of an average size of 1018 bp which represent a total coding surface of 63 % of the whole genome. The genome of *E. ruminantium* strain Welgevonden bears 957 genes of the same average size of 1018 bp. The genome surface of this strain devoted to coding sequences is 62 %. Both genomes comprise 36 transfer RNAs (tRNA) and 3 ribosomal RNAs (rRNA).

**EXAMPLE 2. IDENTIFICATION OF TARGET GENES FOR STRAIN SPECIFIC DIFFERENTIAL DIAGNOSTIC IN THE GARDEL AND WELGEVONDEN STRAINS OF *E. RUMINANTIUM***

The differential analysis of the whole genomes of *E. ruminantium* strains Gardel and Welgevonden showed the presence of coding sequences which are present in only one of the strains and not in the other. Some of the CDS which are unique to *E. ruminantium* strain Gardel and found only in the genome of this strain are presented in Table 1 (Seq ID NO 1 to Seq ID NO 5). One of the CDS which is unique to *E. ruminantium* strain Welgevonden and found only in the genome of this strain is presented in Table 1 (Seq ID NO 6). Since these sequences are unique to one or the other strain, they clearly represent targets for the differential detection of *E. ruminantium* strain Gardel versus *E. ruminantium* strain Welgevonden.

The differential analysis of the whole genomes of *E. ruminantium* strains Gardel and Welgevonden also showed the presence of coding sequences which are affected by one or several mutations in one of the two strains and for which a non-mutated, functionally active and normal allele is present in the genome of the other strain. Mutations yielded a stop codon which may result in shorter but still predicted CDS depending upon the size of the remaining fragments. Truncated genes resulting in a single CDS are denominated partial CDS, whereas those resulting in two or more predicted CDS are described as fragmented CDS. These coding sequences are presented in Table 1. One such CDS in the genome of *E. ruminantium* strain Gardel which is affected by mutations and differs from its native counterpart in *E. ruminantium* strain Welgevonden is presented in Table 1 (SEQ ID NO 7). This is a truncated version of the native gene in *E. ruminantium* strain Welgevonden (Table 1, SEQ ID NO 8). The genome of *E. ruminantium* strain Welgevonden also bears mutated genes, with respect to their allelic variant counterparts in the genome of *E. ruminantium* strain Gardel. Three of these CDS which are affected by mutations generating a truncated version of the genes are presented in Table 1 (SEQ ID NO 9 to SEQ ID NO 12). The native full length allele of these CDS present in the genome of *E. ruminantium* strain Gardel are shown in Table 1 (SEQ ID NO 13 to SEQ ID NO 15). One series of CDS in *E. ruminantium* strain Welgevonden (SEQ ID NO 11 and SEQ ID NO 12), whose native full length alleles are found in the genome of *E. ruminantium* strain Gardel (Table 1, SEQ ID NO 15) was affected by mutations generating a frameshift.
<table>
<thead>
<tr>
<th>Target gene</th>
<th>Gene in Gardel</th>
<th>Status</th>
<th>Gene in Welgevonden</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erum 3</td>
<td>ERGA_CDS_05600 (SEQ ID No 1)</td>
<td>Unique gene</td>
<td>None</td>
<td>Sequence absent (full deletion)</td>
</tr>
<tr>
<td>Erum 5</td>
<td>ERGA_CDS_07600 (SEQ ID No 2)</td>
<td>Unique gene</td>
<td>None</td>
<td>Sequence absent (full deletion)</td>
</tr>
<tr>
<td>Erum 2</td>
<td>ERGA_CDS_04990 (SEQ ID No 3)</td>
<td>Unique gene</td>
<td>None</td>
<td>Partial deletion</td>
</tr>
<tr>
<td>Erum 4</td>
<td>ERGA_CDS_05610 (SEQ ID No 4)</td>
<td>Unique gene</td>
<td>None</td>
<td>Extensive mutations</td>
</tr>
<tr>
<td>Erum 6</td>
<td>None</td>
<td>Partial deletion</td>
<td>ERWE_CDS_08340 (SEQ ID No 5)</td>
<td>Unique gene</td>
</tr>
<tr>
<td>Erum 1</td>
<td>ERGA_CDS_04550 (SEQ ID No 6)</td>
<td>Unique gene</td>
<td>None</td>
<td>Extensive mutations</td>
</tr>
<tr>
<td>Erum 10</td>
<td>ERGA_CDS_07340 (SEQ ID No 7)</td>
<td>Partial deletion</td>
<td>ERWE_CDS_07420 (SEQ ID No 8)</td>
<td>Full length gene</td>
</tr>
<tr>
<td>Erum 7</td>
<td>ERGA_CDS_01350 (SEQ ID No 13)</td>
<td>Full length gene</td>
<td>ERWE_CDS_01390 (SEQ ID No 9)</td>
<td>Partial deletion</td>
</tr>
<tr>
<td>Erum 9</td>
<td>ERGA_CDS_05750 (SEQ ID No 14)</td>
<td>Full length gene</td>
<td>ERWE_CDS_05840 (SEQ ID No 10)</td>
<td>Partial deletion</td>
</tr>
<tr>
<td>Erum 8</td>
<td>ERGA_CDS_04510 (SEQ ID No 15)</td>
<td>Full length gene</td>
<td>ERWE_CDS_04590 (SEQ ID No 11)</td>
<td>Frameshift (partial deletion)</td>
</tr>
</tbody>
</table>

**EXAMPLE 3. DIFFERENTIAL DETECTION OF STRAIN GARDEL AND STRAIN WELGEVONDEN OF E. RUMINANTIUM BASED ON PCR AMPLIFICATION PATTERNS OF THE TARGET GENES**

Differential PCR identification of strains Gardel and Welgevonden of *E. ruminantium* was achieved using primers described in Table 2.
Table 2

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer name</th>
<th>SEQ ID</th>
<th>Orientation</th>
<th>Size (mer)</th>
<th>CDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erum 1</td>
<td>P-Erum 1-A</td>
<td>SEQ ID #16</td>
<td>Sense</td>
<td>21</td>
<td>ERGA_CDS_04350</td>
</tr>
<tr>
<td>Erum 1</td>
<td>P-Erum 1-B</td>
<td>SEQ ID #17</td>
<td>Antisense</td>
<td>21</td>
<td>ERGA_CDS_04350</td>
</tr>
<tr>
<td>Erum 2</td>
<td>P-Erum 2-A</td>
<td>SEQ ID #18</td>
<td>Sense</td>
<td>25</td>
<td>ERGA_CDS_04990</td>
</tr>
<tr>
<td>Erum 2</td>
<td>P-Erum 2-B</td>
<td>SEQ ID #19</td>
<td>Antisense</td>
<td>23</td>
<td>ERGA_CDS_04990</td>
</tr>
<tr>
<td>Erum 3</td>
<td>P-Erum 3-A</td>
<td>SEQ ID #20</td>
<td>Sense</td>
<td>20</td>
<td>ERGA_CDS_05600</td>
</tr>
<tr>
<td>Erum 3</td>
<td>P-Erum 3-B</td>
<td>SEQ ID #21</td>
<td>Antisense</td>
<td>20</td>
<td>ERGA_CDS_05600</td>
</tr>
<tr>
<td>Erum 4</td>
<td>P-Erum 4-A</td>
<td>SEQ ID #22</td>
<td>Sense</td>
<td>19</td>
<td>ERGA_CDS_05610</td>
</tr>
<tr>
<td>Erum 4</td>
<td>P-Erum 4-B</td>
<td>SEQ ID #23</td>
<td>Antisense</td>
<td>22</td>
<td>ERGA_CDS_05610</td>
</tr>
<tr>
<td>Erum 5</td>
<td>P-Erum 5-A</td>
<td>SEQ ID #24</td>
<td>Sense</td>
<td>23</td>
<td>ERGA_CDS_07600</td>
</tr>
<tr>
<td>Erum 5</td>
<td>P-Erum 5-B</td>
<td>SEQ ID #25</td>
<td>Antisense</td>
<td>19</td>
<td>ERGA_CDS_07600</td>
</tr>
<tr>
<td>Erum 6</td>
<td>P-Erum 6-A</td>
<td>SEQ ID #26</td>
<td>Sense</td>
<td>26</td>
<td>ERWE_CDS_08340</td>
</tr>
<tr>
<td>Erum 6</td>
<td>P-Erum 6-B</td>
<td>SEQ ID #27</td>
<td>Antisense</td>
<td>23</td>
<td>ERWE_CDS_08340</td>
</tr>
<tr>
<td>Erum 7</td>
<td>P-Erum 7-A</td>
<td>SEQ ID #28</td>
<td>Sense</td>
<td>25</td>
<td>ERGA_CDS_01350</td>
</tr>
<tr>
<td>Erum 7</td>
<td>P-Erum 7-B</td>
<td>SEQ ID #29</td>
<td>Antisense</td>
<td>25</td>
<td>ERGA_CDS_01390</td>
</tr>
<tr>
<td>Erum 8</td>
<td>P-Erum 8-A</td>
<td>SEQ ID #30</td>
<td>Sense</td>
<td>25</td>
<td>ERWE_CDS_04590</td>
</tr>
<tr>
<td>Erum 8</td>
<td>P-Erum 8-B</td>
<td>SEQ ID #31</td>
<td>Antisense</td>
<td>25</td>
<td>ERWE_CDS_04600</td>
</tr>
<tr>
<td>Erum 9</td>
<td>P-Erum 9-A</td>
<td>SEQ ID #32</td>
<td>Sense</td>
<td>25</td>
<td>ERWE_CDS_05750</td>
</tr>
<tr>
<td>Erum 9</td>
<td>P-Erum 9-B</td>
<td>SEQ ID #33</td>
<td>Antisense</td>
<td>25</td>
<td>ERWE_CDS_05840</td>
</tr>
<tr>
<td>Erum 10</td>
<td>P-Erum 10-A</td>
<td>SEQ ID #34</td>
<td>Sense</td>
<td>25</td>
<td>ERGA_CDS_07340</td>
</tr>
<tr>
<td>Erum 10</td>
<td>P-Erum 10-B</td>
<td>SEQ ID #35</td>
<td>Antisense</td>
<td>25</td>
<td>ERWE_CDS_07420</td>
</tr>
</tbody>
</table>

DNA is extracted from elementary bodies of *E. ruminantium*, as described by Perez *et al.* (1997). *E. ruminantium* strains are grown in BUEC cells as described above. Elementary bodies are purified from the culture supernatant by differential centrifugation and resuspended in 350 µl of PBS to which is added 150 µl of buffer containing 25 mM Tris-HCl (pH 8.0), 10 mM MgCl₂ and 125 µg of DNase in order to remove contaminating host cell DNA. After incubation for 90 min. at 37°C, the reaction is stopped by addition of 25 mM EDTA. Elementary bodies are washed three times in water and lysed by overnight incubation at 55°C in a solution of 100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 25 mM EDTA, 1.5% SDS and 250 µg/ml of proteinase K. Bacterial DNA is extracted with phenol-chloroform,
precipitated with cold ethanol and resuspended in sterile distilled water. Contamination with cell DNA is evaluated by slot blot hybridization using labeled bovine DNA as a probe and dilutions of bovine DNA (12.5 ng and 25 ng) as positive controls.

PCR amplification of amplicons is performed by mixing 250 ng of E. ruminantium DNA, 2.5 U of Taq DNA polymerase, 200 nM of each dNTP, 1 µM of each, sense and antisense, primer and 3 mM MgCl₂ in a final volume of 50 µl. Amplification is done under the following conditions: 5 min denaturation at 94°C, followed by 30 cycles of amplification with a 1-min denaturation, 45 sec of annealing at 45°C and 2 min extension at 72°C. An extra extension step of 10 min at 72°C is added after completion of the 30 cycles. PCR products, i.e. amplicons, are analysed by 1% agarose gel electrophoresis in Tris-borate-EDTA buffer.

The results are summarized in Table 3, Figure 1, and Figure 2.

Legend of Figure 1:
1: Molecular weight marker (100-bp ladder); 2: Erga with primers P-Erum 1-A + P-Erum 1-B; 3: Erwe with primers P-Erum 1-A + P-Erum 1-B; 4: Control sample with primers P-Erum 1-A + P-Erum 1-B; 5: Erga with primers P-Erum 2-A + P-Erum 2-B; 6: Erwe with primers P-Erum 2-A + P-Erum 2-B; 7: Control sample with primers P-Erum 2-A + P-Erum 2-B; 8: Erga with primers P-Erum 3-A + P-Erum 3-B; 9: Erwe with primers P-Erum 3-A + P-Erum 3-B; 10: Control sample with primers P-Erum 3-A + P-Erum 3-B; 11: Erga with primers P-Erum 4-A + P-Erum 4-B; 12: Erwe with primers P-Erum 4-A + P-Erum 4-B; 13: Control sample with primers P-Erum 4-A + P-Erum 4-B; 14: Erga with primers P-Erum 5-A + P-Erum 5-B; 15: Erwe with primers P-Erum 5-A + P-Erum 5-B; 16: Control sample with primers P-Erum 5-A + P-Erum 5-B; 17: Erga with primers P-Erum 6-A + P-Erum 6-B; 18: Erwe with primers P-Erum 6-A + P-Erum 6-B; 19: Control sample with primers P-Erum 6-A + P-Erum 6-B; 20: Molecular weight marker (1 EcoRI-HindIII).

Legend of Figure 2:
1: Molecular weight marker (100-bp ladder); 2: Erga with primers P-Erum 7-A + P-Erum 7-B; 3: Erwe with primers P-Erum 7-A + P-Erum 7-B; 4: Control sample with primers P-Erum 7-A + P-Erum 7-B; 5: Erga with primers P-Erum 9-A + P-Erum 9-B; 6: Erwe with primers P-Erum 9-A + P-Erum 9-B; 7: Control sample with primers P-Erum 9-A + P-Erum 9-B; 8: Erga with primers P-Erum 10-A + P-Erum 10-B; 9: Erwe with primers P-Erum 10-A + P-Erum 10-B; 10: Control sample with primers P-Erum 10-A + P-Erum 10-B; 11: Molecular weight marker (1 EcoRI-HindIII); 12: Molecular weight marker (100-bp ladder); 13: Erga with primers P-Erum 8-A + P-Erum 8-B; 14: Erwe with primers P-Erum 8-A + P-Erum 8-B; 15: Control sample with primers P-Erum 8-A + P-Erum 8-B

As shown in Table 3, Fig.1 and Fig. 2, all the pairs of primers described in Table 3 allowed for differential indentification and discrimination of strains Gardel and Welgevonden. The PCR reactions yielded the results expected from in silico prediction of
amplicons (Table 3). The pairs P-Erum 1-A + P-Erum 1-B, P-Erum 2-A + P-Erum 2-B, P-Erum 3-A + P-Erum 3-B, P-Erum 4-A + P-Erum 4-B, P-Erum 5-A + P-Erum 5-B, which target unique genes present only in strain Gardel generated the expected unique amplicons of 172 bp, 217 bp, 508 bp, 642 bp and 238 bp, respectively, on strain Gardel while generating no bands on strain Welgevonden (Table 3, Fig. 1). The pair of primers P-Erum 6-A + P-Erum 6-B which target a unique gene only present in strain Welgevonden yielded, as expected, a single amplicon of 127 bp while no PCR product was obtained on strain Gardel (Table 3, Fig. 1). The pairs P-Erum 7-A + P-Erum 7-B, P-Erum 8-A + P-Erum 8-B, P-Erum 9-A + P-Erum 7-B and P-Erum 10-A + P-Erum 10-B targeting the truncated genes yielded PCR products of the respective expected size of 2791 bp, 552 bp + 1071 bp, 1361 bp and 1095 bp on strain Gardel and 2395 bp, 492 bp, 1178 bp and 1691 bp on strain Welgevonden, respectively (Table 3, Fig. 2). An additional band of 480 bp is observed on strain Gardel with the pair P-Erum 8-A + P-Erum 8-B. This additional band is most likely due to a single low specificity response occurring in Erga.

The primer pairs P-1350-A + P-1350-B, P-4510-A + P-4510-B, P-5750-A + P-5750-B and P-7420-A + P-7420-B yielded PCR products of the respective expected size of 2791, 552+1071, 1361 and 1095 on strain Gardel and 2395, 492, 1178 and 1691 on strain Welgevonden, respectively (Table 3, Fig. 1 and Fig. 2). An additional band of 480 bp is observed on strain Gardel with the pair P-4510-A + P-4510-B. This additional band is most likely due to a single low specificity response occurring in strain Gardel.

Table 3. Strain-specific differential PCR screening of *E. ruminantium* strain Gardel and strain Welgevonden

<table>
<thead>
<tr>
<th>Primer combination</th>
<th><em>E. ruminantium</em> Gardel</th>
<th><em>E. ruminantium</em> Welgevonden</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Erum 1-A + P-Erum 1-B</td>
<td>172</td>
<td>172</td>
</tr>
<tr>
<td>P-Erum 2-A + P-Erum 2-B</td>
<td>217</td>
<td>217</td>
</tr>
<tr>
<td>P-Erum 3-A + P-Erum 3-B</td>
<td>508</td>
<td>508</td>
</tr>
<tr>
<td>P-Erum 4-A + P-Erum 4-B</td>
<td>642</td>
<td>642</td>
</tr>
<tr>
<td>P-Erum 5-A + P-Erum 5-B</td>
<td>238</td>
<td>238</td>
</tr>
<tr>
<td>P-Erum 6-A + P-Erum 6-B</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>P-Erum 7-A + P-Erum 7-B</td>
<td>2791</td>
<td>2791</td>
</tr>
<tr>
<td>P-Erum 8-A + P-Erum 8-B</td>
<td>552+1071</td>
<td>552+1071+480</td>
</tr>
<tr>
<td>P-Erum 9-A + P-Erum 9-B</td>
<td>1361</td>
<td>1361</td>
</tr>
<tr>
<td>P-Erum 10-A + P-Erum 10-B</td>
<td>1095</td>
<td>1095+300</td>
</tr>
</tbody>
</table>

**EXAMPLE 4: DIFFERENTIAL STRAIN-SPECIFIC PCR DETECTION AND IDENTIFICATION OF STRAINS OF *E. RUMINANTUM* DIFFERENT THAN STRAIN GARDEL AND STRAIN WELGEVONDEN**

The use primers listed in Table 2 were used for the specific identification and discrimination of *E. ruminantium* strains other than strain Gardel and strain Welgevonden. The strains others than Gardel and Welgevonden presented in this example are strains Umpala (Mozambique), Senegal (Senegal), Bankouma (Burkina Faso), Bekuy (Burkina Faso), Lamba (Burkina Faso), Banan 1 (Burkina Faso) and Banan 2 (Burkina Faso).
These strains are presented here to illustrate samples from different parts of Sub-Saharan Africa and the Caribbean.

DNA is extracted from elementary bodies of *E. ruminantium* and PCR amplification performed as described in Example 3.

The results are shown in Table 4, Fig. 3 and Fig. 4:

**Legend of Figure 3:**

A. PCR detection with primers P-Erum 1-A + P-Erum 1-B
B. PCR detection with primers P-Erum 3-A + P-Erum 3-B
C. PCR detection with primers P-Erum 2-A + P-Erum 2-B
D. PCR detection with primers P-Erum 4-A + P-Erum 4-B
E. PCR detection with primers P-Erum 6-A + P-Erum 6-B
F. PCR detection with primers P-Erum 5-A + P-Erum 5-B

MW1: Molecular weight marker (100pb DNA ladder); MW2: Molecular weight marker (I HindIII/EcoRI); 1: Strain Senegal attenuated (Satt); 2: Strain Gardel CTVM; 3: Strain Bankouma; 4: Strain Bekuy; 5: Strain Lamba; 6: Strain Banan 1; 7: Strain Banan 2; NC: Negative control; G. Strain Gardel; W: Strain Welgevonden.

**Legend of Figure 4:**

A. PCR detection with primers P-Erum 7-A + P-Erum 7-B
B. PCR detection with primers P-Erum 8-A + P-Erum 8-B
C. PCR detection with primers P-Erum 9-A + P-Erum 9-B
D. PCR detection with primers P-Erum 10-A + P-Erum 10-B

MW1: Molecular weight marker (100pb DNA ladder); MW2: Molecular weight marker (I HindIII/EcoRI); 1: Strain Bankouma; 2: Strain Bekuy; 3: Strain Lamba; 4: Strain Banan 1; 5: Strain Banan 2; 6: Strain Gardel attenuated (Gatt); 7: Strain Gardel CTVM; 8: Strain Senegal; 9: Strain Senegal attenuated (Satt); NC: Negative control; G. Strain Gardel; W: Strain Welgevonden.

As shown in Table 4, Fig. 3 and Fig. 4, the combined use of all the pairs of primers described in Table 2 allowed for differential identification and discrimination of strains other than strains Gardel and Welgevonden. The PCR reactions results are summarized in Table 4. The pairs P-Erum 1-A + P-Erum 1-B, P-Erum 2-A + P-Erum 2-B, P-Erum 3-A + P-Erum 3-B, P-Erum 4-A + P-Erum 4-B, P-Erum 5-A + P-Erum 5-B which target unique genes present only in strain Gardel and the pair P-Erum 6-A + P-Erum 6-B which targets a unique gene only present in strain Welgevonden all yielded differing patterns of PCR products depending on the strain (Table 4, Fig 3). Differing patterns depending upon the strain were also observed using the pairs P-Erum 7-A + P-Erum 7-B, P-Erum 8-A + P-Erum 8-B, P-Erum 9-A + P-Erum 7-B and P-Erum 10-A + P-Erum 10-B which target the truncated genes (Table 4, Fig. 3).
It is however the overall analysis of all the PCR patterns yielded by all the pairs of primers described in Table 2 which provides a strain specific diagnostic. The strains Bekuy and Lamba which were isolated in Burkina Faso from the nearby villages of Bekuy and Lamba, respectively, are most likely to be two isolates of the same strain. Furthermore, these strains display the same map-1 genotype determined by PCR amplification and sequencing of the map-1 gene. All the other strains display differing map-1 genotypes. This further indicates that strains Bekuy and Lamba are two isolate of the same strain. The identical overall pattern obtained for these two strains with all the pairs of primers described in Table 2 also further demonstrate the strain-specificity of the subject of the invention and its ability to identify different strains and separate isolates of the same strain.
Table 4: Strain-specific differential PCR screening of *E. ruminantium*

<table>
<thead>
<tr>
<th>Primer combination</th>
<th>Gardel</th>
<th>Welgevonden</th>
<th>Umpala</th>
<th>Senegal</th>
<th>Bankouma</th>
<th>Bekuy</th>
<th>Lamba</th>
<th>Banan1</th>
<th>Banan2</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Erum 1-A + P-Erum 1-B</td>
<td>172</td>
<td>None</td>
<td>172</td>
<td>172</td>
<td>Multibands</td>
<td>172</td>
<td>172</td>
<td>172</td>
<td>Multibands</td>
</tr>
<tr>
<td>P-Erum 2-A + P-Erum 2-B</td>
<td>217</td>
<td>None</td>
<td>515</td>
<td>500 + 900</td>
<td>280+500+1200</td>
<td>500+900</td>
<td>500+900</td>
<td>500+900</td>
<td>500+1200</td>
</tr>
<tr>
<td>P-Erum 3-A + P-Erum 3-B</td>
<td>508</td>
<td>None</td>
<td>508</td>
<td>None</td>
<td>560</td>
<td>508+1900</td>
<td>508+1900</td>
<td>None</td>
<td>508</td>
</tr>
<tr>
<td>P-Erum 4-A + P-Erum 4-B</td>
<td>642</td>
<td>None</td>
<td>642</td>
<td>642</td>
<td>642&lt;sup&gt;a&lt;/sup&gt;</td>
<td>642</td>
<td>642</td>
<td>642</td>
<td>642</td>
</tr>
<tr>
<td>P-Erum 5-A + P-Erum 5-B</td>
<td>238</td>
<td>None</td>
<td>238</td>
<td>None</td>
<td>238</td>
<td>238</td>
<td>238</td>
<td>238</td>
<td>238</td>
</tr>
<tr>
<td>P-Erum 6-A + P-Erum 6-B</td>
<td>None</td>
<td>127</td>
<td>None</td>
<td>127</td>
<td>None</td>
<td>127</td>
<td>127</td>
<td>127</td>
<td>None</td>
</tr>
<tr>
<td>P-Erum 7-A + P-Erum 7-B</td>
<td>2791</td>
<td>2395</td>
<td>2791+ 500</td>
<td>2395</td>
<td>2395</td>
<td>2395</td>
<td>2395</td>
<td>2395</td>
<td>None</td>
</tr>
<tr>
<td>P-Erum 8-A + P-Erum 8-B</td>
<td>552+1071 +480</td>
<td>492</td>
<td>1200 + 500&lt;sup&gt;a&lt;/sup&gt;</td>
<td>492</td>
<td>492</td>
<td>492</td>
<td>492</td>
<td>492</td>
<td>552+1071 +480</td>
</tr>
<tr>
<td>P-Erum 9-A + P-Erum 9-B</td>
<td>1361</td>
<td>1178</td>
<td>1361</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1178+1361</td>
</tr>
<tr>
<td>P-Erum 10-A + P-Erum 10-B</td>
<td>1095+300</td>
<td>1691</td>
<td>820</td>
<td>820</td>
<td>820</td>
<td>820</td>
<td>820</td>
<td>820</td>
<td>1095</td>
</tr>
</tbody>
</table>

<sup>a</sup>: presence of additional multiple bands is observed
EXAMPLE 5: DIFFERENTIAL SPECIFIC PCR DETECTION AND IDENTIFICATION OF ATTENUATED AND DIFFERING DERIVATES OF STRAINS GARDEL AND SENEGAL

The primers listed in Table 2 also allow the specific identification of attenuated variants of known strains of *E. ruminantium*.

The following variants were tested:
- attenuated derivates of strains Gardel and Senegal denominated Gatt (for Gardel-attenuated) and Satt (for Senegal-attenuated), respectively. The strain Gatt was obtained from the virulent strain Gardel through 248 successive passages BUEC cells whereas strain Satt was obtained from the virulent strain Senegal following 64 passages on BUEC cells. Both the Gatt and Satt strains display an attenuated phenotype characterized by a lack of virulence.
- strain Gardel CTVM, which is a subset of strain Gardel maintained in a differing cell environment, and was reported has having undergone mutations in the *map1* operon and displaying a diverging phenotype (Bekker et al., 2004).

DNA is extracted from elementary bodies of *E. ruminantium* and PCR amplification performed as described in Example 3.

The results are shown in Table 5, Figure 4, and Figure 5.

**Legend of Figure 4:**

A. PCR detection with primers P-Erum 7-A + P-Erum 7-B
B. PCR detection with primers P-Erum 8-A + P-Erum 8-B
C. PCR detection with primers P-Erum 9-A + P-Erum 9-B
D. PCR detection with primers P-Erum 10-A + P-Erum 10-B

MW1: Molecular weight marker (100pb DNA ladder); MW2: Molecular weight marker (1 HindIII/EcoRI); 1: Strain Bankouma; 2: Strain Bekuy; 3: Strain Lamba; 4: Strain Banan 1; 5: Strain Banan 2; 6: Strain Gardel attenuated (Gatt); 7: Strain Gardel CTVM; 8: Strain Senegal; 9: Strain Senegal attenuated (Satt); NC: Negative control; G: Strain Gardel; W: Strain Welgevonden.

**Legend of Figure 5:**

- A. PCR analysis of virulent and attenuated strains with primers P-Erum 1-A + P-Erum 1-B
MW1: Molecular weight marker (100-pb DNA ladder); MW2: Molecular weight marker (1 HindIII/EcoRI); 1: Strain Gardel attenuated (Gatt); 2: Strain Gardel; 3: Negative control.
- B. PCR analysis of virulent and attenuated strains with primers P-Erum 2-A + P-Erum 2-B and P-Erum 6-A + P-Erum 6-B
MW1: Molecular weight marker (100-pb DNA ladder); MW2: Molecular weight marker (1 HindIII/EcoRI); Analysis with P-Erum 2-A + P-Erum 2-B of 1: Strain Gardel attenuated (Gatt); 2: Strain Gardel; 3: Negative control; Analysis with P-Erum 6-A + P-Erum 6-B of 4:
Strain Gardel attenuated (Gatt); 5: Strain Gardel; 6: Strain Welgevonden; 3: Negative control.

- C. PCR analysis of virulent and attenuated strains with primers P-Erum 3-A + P-Erum 3-B, P-Erum 4-A + P-Erum 4-B and P-Erum 5-A + P-Erum 5-B

MW1: Molecular weight marker (100-pb DNA ladder); MW2: Molecular weight marker (HindIII/EcoRI); Analysis with P-Erum 4-A + P-Erum 4-B of 1: Strain Gardel attenuated (Gatt); 2: Strain Gardel; 3: Negative control; Analysis with P-Erum 5-A + P-Erum 5-B of 4: Strain Gardel attenuated (Gatt); 5: Strain Gardel; 6: Negative control; Analysis with P-Erum 3-A + P-Erum 3-B of 7: Strain Gardel attenuated (Gatt); 8: Strain Gardel; 9: Strain Gardel; 10: Negative control.

As shown in Table 5, Fig. 4 and Fig. 5, the combined use of all the pairs of primers described in Table 2 also allowed for the differential identification and discrimination of variants and attenuated derivatives of known strains. The PCR reactions results are summarized in Table 5. The overall PCR patterns generated on the strain Gardel and and two of its derivates, the strain Gardel CTVM and the attenuated strain Gatt show the presence of slight variations –Table 5, Fig. 4, Fig. 5). Furthermore, each strain is characterized by a specific pattern. The strain Gatt differs from the parental virulent strain Gardel by the products from primers pairs P-Erum 6-A + P-Erum 6-B and P-Erum 7-A + P-Erum 7-B, whereas the strain Gardel CTVM differs from the parental strain Gardel by the product from the primers pair P-Erum 6-A + P-Erum 6-B. Similarly, the primers pair P-Erum 7-A + P-Erum 7-B allows for discrimination between the strain Gardel CTVM and the attenuated Gardel strain Gatt. A similar situation is observed between the parental virulent strain Senegal and its attenuated derivative Satt (Table 5, Fig. 4). The virulent strain Senegal and the attenuated strains Satt differ by the PCR product from the primer pairs P-Erum 2-A + P-Erum 2-B, P-Erum 3-A + P-Erum 3-B, P-Erum 6-A + P-Erum 6-B and P-Erum 7-A + P-Erum 7-B.
Table 5: Differential identification of attenuated Gardel and Senegal strains of *E. ruminantium*

<table>
<thead>
<tr>
<th>Primer combination</th>
<th>Gardel (Virulent)</th>
<th>Gatt (attenuated Gardel)</th>
<th>Gardel CTVM</th>
<th>Senegal</th>
<th>Satt (attenuated Senegal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Erum 1-A + P-Erum 1-B</td>
<td>172</td>
<td>172</td>
<td>172</td>
<td>172</td>
<td>172</td>
</tr>
<tr>
<td>P-Erum 2-A + P-Erum 2-B</td>
<td>217</td>
<td>217</td>
<td>217</td>
<td>500 + 900</td>
<td>500 + 900+210</td>
</tr>
<tr>
<td>P-Erum 3-A + P-Erum 3-B</td>
<td>508</td>
<td>508</td>
<td>508</td>
<td>None</td>
<td>508+1900</td>
</tr>
<tr>
<td>P-Erum 4-A + P-Erum 4-B</td>
<td>642</td>
<td>642</td>
<td>642</td>
<td>642</td>
<td>642</td>
</tr>
<tr>
<td>P-Erum 5-A + P-Erum 5-B</td>
<td>238</td>
<td>238</td>
<td>238</td>
<td>None</td>
<td>238</td>
</tr>
<tr>
<td>P-Erum 6-A + P-Erum 6-B</td>
<td>None</td>
<td>127</td>
<td>127</td>
<td>127</td>
<td>127</td>
</tr>
<tr>
<td>P-Erum 7-A + P-Erum 7-B</td>
<td>2791</td>
<td>None</td>
<td>2791</td>
<td>2395</td>
<td>2395+700+300</td>
</tr>
<tr>
<td>P-Erum 8-A + P-Erum 8-B</td>
<td>552+1071+480</td>
<td>552+1071+480</td>
<td>552+1071+480</td>
<td>492</td>
<td>492</td>
</tr>
<tr>
<td>P-Erum 9-A + P-Erum 9-B</td>
<td>1361</td>
<td>1361</td>
<td>1361</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>P-Erum 10-A + P-Erum 10-B</td>
<td>1095+300</td>
<td>1095+300</td>
<td>1095+300</td>
<td>820</td>
<td>820</td>
</tr>
</tbody>
</table>

EXAMPLE 6: ABSENCE OF CROSS-REACTION WITH OTHER RICKETTSIALES

To verify the specificity assessment of the primers listed in Table 2, they were tested on *Rickettsiales* belonging to other species and genera than *E. ruminantium* i.e. *Ehrlichia canis*, *Anaplasma platys* and *Anaplasma marginale*.

DNA extraction and PCR amplification were performed as described in Example 3.

The results are shown in Figures 6, 7, and 8.

**Legend of Figure 6:**

- A. PCR detection with probe EHR16S specific to *Ehrlichia spp.* 16S rDNA
  - MW: Molecular weight marker (100-bp ladder); 1, 2 and 3: DNA isolated from blood samples from dogs infected with *Anaplasma platys*, 4, 5 and 8: DNA isolated from blood samples from dogs infected with *Ehrlichia canis*; 6: *Ehrlichia canis* positive control; 7: Negative control; 9 and 10: Control DNA isolated from blood samples from non-infected dogs.

- B. PCR detection with Nested PCR probes specific to *Anaplasma platys*
  - MW: Molecular weight marker (100-bp ladder); 1, 2 and 3: DNA isolated from blood samples from dogs infected with *Anaplasma platys*. 
- C. PCR detection with Nested PCR probes specific to *Ehrlichia canis*

MW: Molecular weight marker (100-bp ladder); 4, 5 and 8: DNA isolated from blood samples from dogs infected with *Ehrlichia canis*; 11: DNA from canine monocytes cultures infected with *E. canis* (supernatant); 12: DNA from canine monocytes cultures infected with *E. canis* (pellet).

Legend of Figure 7:

A. PCR detection with primers P-Erum 1-A + P-Erum 1-B
B. PCR detection with primers P-Erum 2-A + P-Erum 2-B
C. PCR detection with primers P-Erum 3-A + P-Erum 3-B
D. PCR detection with primers P-Erum 4-A + P-Erum 4-B
E. PCR detection with primers P-Erum 5-A + P-Erum 5-B
F. PCR detection with primers P-Erum 6-A + P-Erum 6-B

MW1: Molecular weight marker (100pb DNA ladder); MW2: Molecular weight marker (1 HindIII/EcoRI); 1, 2 and 3: DNA isolated from blood samples from dogs infected with *Anaplasma platys*, 4, 5 and 8: DNA isolated from blood samples from dogs infected with *Ehrlichia canis*; 9 and 10: Control DNA isolated from blood samples from non-infected dogs, 11: DNA from canine monocytes cultures infected with *E. canis* (supernatant); 12: DNA from canine monocytes cultures infected with *E. canis* (pellet); Am: DNA from *Anaplasma marginale*; G: DNA from strain Gardel; NC: Negative control.

Legend of Figure 8:

A. PCR detection with primers P-Erum 7-A + P-Erum 7-B
B. PCR detection with primers P-Erum 8-A + P-Erum 8-B
C. PCR detection with primers P-Erum 9-A + P-Erum 9-B
D. PCR detection with primers P-Erum 10-A + P-Erum 10-B

MW1: Molecular weight marker (100pb DNA ladder); MW2: Molecular weight marker (1 HindIII/EcoRI); 1, 2 and 3: DNA isolated from blood samples from dogs infected with *Anaplasma platys*, 4, 5 and 8: DNA isolated from blood samples from dogs infected with *Ehrlichia canis*; 9 and 10: Control DNA isolated from blood samples from non-infected dogs, 11: DNA from canine monocytes cultures infected with *E. canis* (supernatant); 12: DNA from canine monocytes cultures infected with *E. canis* (pellet); Am: DNA from *Anaplasma marginale*; G: DNA from strain Gardel; W: DNA from strain Welgevonden; NC: Negative control.

Fig. 6 indicates that the samples used indeed contain DNA from *A. platys* and *E. canis* as demonstrated by their recognition by 16S rDNA-specific primers and primers for nested PCR specific to each species. As shown in Fig. 7 and Fig. 8, the pairs of primers described in Table 2 are strictly specific to *E. ruminantium* and display no cross-reaction with other related *Rickettsiales* since no specific PCR product could be detected on *E. canis*, *A. platys* and *A. marginale* (Fig. 7 and Fig. 8). Whereas no PCR products are detectable on
whatever pair of primers was used, PCR products were visible on *A. platys* and *E. canis*. However, all these PCR products are generated by cross-reactions with canine blood cells as shown by the detection of these same bands on non-infected canine cells (Fig. 7 and Fig. 8). This demonstrate that the primers described in Table 2 and targeting the target genes described in Table 1 allow for specific identification of *E. ruminantium* and discrimination between strains of *E. ruminantium* even when other *Rickettsiales* are present.

The tools provided by the invention allow thus both for specific detection of *E. ruminantium*, even in presence of contaminating related *Rickettsiales*, for specific discrimination between different strains of *E. ruminantium* and for specific discrimination between a virulent strains and its vaccinal attenuated derivates. This in turn allows for monitoring of vaccination.
CLAIMS

1) Use of the following set of target genes:

- Erum1, defined by the sequence SEQ ID NO: 6
- Erum2, defined by the sequence SEQ ID NO: 3
- Erum3, defined by the sequence SEQ ID NO: 1
- Erum4, defined by the sequence SEQ ID NO: 4
- Erum5, defined by the sequence SEQ ID NO: 2
- Erum6, defined by the sequence SEQ ID NO: 5
- Erum7, defined by the sequence SEQ ID NO: 13
- Erum8, defined by the sequence SEQ ID NO: 15
- Erum9, defined by the sequence SEQ ID NO: 14
- Erum10, defined by the sequence SEQ ID NO: 8,

for the strain-specific detection of *Ehrlichia ruminantium*.

2) A method for the strain-specific detection of *Ehrlichia ruminantium* wherein said method comprises detecting, for each of the genes Erum1 to Erum10, whether an allele of said gene is present in the bacteria to be tested, and determining the form of said allele.

3) A method of claim 2, which comprises performing PCR amplification of all the target genes Erum1 to Erum10, and checking, for each of these genes, the presence of one or more amplification product(s), and the size of said amplification product(s).
Figure 7
Figure 8
SEQUENCE LISTING

 Target Genes for Strain-Specific Diagnostic of Ehrlichia Ruminantium and Use Thereof

 MJP/mad-F1367-12/WO

 35

 PatentIn version 3.3

 1

 630

 DNA

 Ehrlichia ruminantium

 60

 120

 180

 240

 300

 360

 420

 480

 540

 600

 630

 Ehrlichia ruminantium

 atgaaaggat ctttatctgc taaagttatt tctgaaaatc taccattagt agagatggaa  

 aaacgcagtct ttagtccctc tgctcgatt ttttctcacta atcataagtt gggacctgtc  

 atggaccttg gaatttatatat caataataat ctagtaatc ttggtttatt aacgaaggaa  

 aaccttttata ctgtaatgaa cctaagaata aattggtaaag tgtgcttttg taaacctttg  

 tctataggca atgcataaca tacagtacat atgtacttta atgaactgta agcttttaaaa  

 gaatcgagg gaattgaaaa tgcgtgatct caacacgtac gttcggacac ccccttgcat  

 aacatactct tctaaactata gaataacatga tttacaataac gtctcataata tgtcataaca  

 ctattagtgtt cagtatatt tatacgttca aataatacc gcagtaagag gaataacttacc ccaatatcaca  

 gcaatatcsc ataatagaaga agaagaaggt gcagtaagag gaataacttacc ccaatatcaca  

 cttcctaagt cccagcagc actggcggttt ttctgcttttt tctcagatatg tgggtaa  

 tttgaacat ttagcagaag ataccattaa

 2

 303

 DNA

 Ehrlichia ruminantium

 60

 120

 180

 atggatttaa aataaactata aaagagatag ttgattttcat tttgtaatgat taattttgtt  

 aataggtttt ttatgtaatat caaaagttaa agctttcatc ccccttgcat  

 atggatattat attatattat attatattat  

 cttcctataaat cagatgatga attatatattat gcccctttgatttttttttttgtgctattcaaatgctttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
ggatatataa taccttgagga ttttatgttc tcagaacaaa tcgtaggtag agaaaatgca 240
gaaatgcttt caatatcaga tacagaggg ggggggggag agcttagtag aagaaaattc 300
tag 303

<210> 3
<211> DNA
<213> Ehrlichia ruminantium

<400> 3
atgtatttag ctattttagt agctggtttt gtggtaacct atagtaatta tcgagatat
aattatgata aaaacctgct tttcttttct tctaggggag aagatgatga atataaatat
120
gtccctagga aagagagaga taatatcaat ttatatctata taatttttagt tagtgtaag
180
ttaaatttgc aagagatata tgccttaatt gatgtatatag aagatatag aatatttttag
240
gcaaatatgt ttaaatgttt ttatatatttaa 270

<210> 4
<211> DNA
<213> Ehrlichia ruminantium

<400> 4
gtgctttacct taatgggtta aatattttagt ttgagatataa tttcttcttttaa tactaaagaa 60
ttccaatagc cagctttaaa ttgctttacgt cataaagat tatattgcata tagctacacgtt
120
tgtataggac atatagacca aacaaacact tctacacgtt tatcataagt taattttatctt
180
eaatatttgat tggctttcata gagtttacag taattttact ttcacattata tgcctttgat
240
gataagagag tataactcgaac tcgagaaata agagatagta tgcaggtcagttgctcgtcgc 300
aaaataacc gcctttgccttt taattactta atcagatata aataaatcact 360
tatatataaaataaaacttaaagtttaataa atagataataa aataaatcact 420
aaaataatgttg gacagtgtgctt cctttatata tttttattcg 480

tttcttcat tagacttcacct taatataaag tttaagagcctt tggaggattg 540
caaatgcagt tatttacagc atagataaact attcttattcct ttaataatattttaa tattcattca
600
gaggtgattc cactggttag ttaataaatag tttttattcg aataaatatcact 660
accatggtag ttacttcat aggataaaa aagagagag gcagagactaatt aatatcact 720
gaggtgattc aacactctac ctcctttctttt actcagacat caggataaaact 780
attaatgtag tttctttc ttagatataa atatatgtaga cttcttttgttacct 828

<210> 5
<210> 6
<211> 186
<212> DNA
<213> Ehrlichia ruminantium

<400> 6
atgatccaca gtttatattgga gttaaacca atcaattatt acgaatatat cgaatatatat 60
acaatatcat tgtaacaca tatcaataat ttataacca aatataagag aaaaattatt 120
ataactctgc ttaataaect atttaaactt attacttttt gtgatgttgg gttgataa 180
caatga 186

<210> 7
<211> 3522
<212> DNA
<213> Ehrlichia ruminantium

<400> 7
atgaatgaga taatccctata cacaggcagt tcgctgtttttt atatatggt atcctagtt 60
cgtctgttgg ttaggttttg atgttatgtg ttgaggtta taagaatataa gtaaagaa 120
ttgacactat cagataatta tacaagag gggagttact attgtagctc ggtgaat 180
gaaaaagtac aagggactcc ataattgcta attacttttt tataacattt aaaaatcgtt 240
taccataatg ataggtgta tataagttat gttaaataaat ccaaatctac ttttccttct 300
acccaaattgg atccagtaac acataagtt cctggcgttc aactatata aacaaagtt 360
gcgatagaa gtcaccaagt tatggataag gaaaactgtg gttggtggt tcactata 420
catatagaa ctatctataaa aacaagctt atgttttaaat ctgatggttt gggtgattgt 480
aaggtatatt cgttgagagaa aaagtagta ctcgaaatctct ttaagaat aaaaatat 540
aagagagag tttttgtaag tggtagatt ctaaatgtga taaaaaggt tcaagtttaa 600
ttacatggt ctgttattgaa aaacaggat gagaagagtt atgggtctag tagcgtggt 660
acaggtctg ttaaggtat atctgttag gasaaagtag taccctgaagg tgataacta 720
agaataaatg aaaaaaagag aagatgtttt gtaagttgtc gttgcaaac ttggtgtatg 780
aaaagtgatc aagttaaatt atctggttct agattagaaa aacttagatga gagaagaagat  840
gttcagctaa caggtgtgac aggtagact aaggacaaat ctgtagagaag aaaaagttgta  900
tctgaaggtta ctgctataag agatgaaaag gagaagtagtg ttgctagaaa tgttgggttt  960
acttttaacct tccaaagttgg taatgtaaaag gatgtaaaag taatacttta aggtgttagt 1020
ttaggtttaaa tagaggattc agttttatct gctttctagtgt tggaactacag tggtaaggat 1080
aaataagcttg ttatatgtgt tggacaaagaa agtagcttttc aatattttgctt aaagtttgagat 1140
ttgtttacta ctgttgaaga tagttcaaga aataattctgt gtttaagtgta aacttttgcttct 1200
ttaatgttaggtt attttgacag aaattttgtatat ccttgtatatt aagagcgaacag taagtaagttta 1260
gttgcttagtt tcgtcctgta taattttata tatacacttaa aagaaataacag ttggtgctttta 1320
gatctcctct aatgcaagag tcaaccttttctactagttata ctaatgtgca tagctaatatat 1380
gatgtgacttg aaatagttgt aagtttatatt ccgctgtgatt ttggttctgta tggtagaatga 1440
caagctaaac aagaaagagag taatcactacag ggtgttctga tgatatataaa 1500
gcgctgctgc atagtcactgt tggatgtgact cagaaacagc taagttagat cagactgattatg 1560
ttggttctctg atgtatataa aacaagctaaa cagagttgaag atactaacttgg gggtgcttttt 1620
atatgctcag gttctcataac ccggtgtgctgc cctgtaattat ccagttgaaatgg taaatgttagt 1680
gttggtgtat atcgagttga ctagttttctc gataaatataa aacaagctaa acaagaaaa 1740
gatactaaagc aggggtcttttt tagagctaca gttctcataac ccgctggctgac gcatagtcaca 1800
tatgatatga tctggaaataag cttcactgtga tgcagctagtt actttagttcct tggatgtgta 1860
aacaactaca aacagcactga aagactactaag ccggtgtgcttt ttagtttcaag agttttctgta 1920
tctgctaatgt tagatattttgt tggagttgatt agtttaaggag aaaaangctagattt gataatgtgaa 1980
aaggtttgtta agttcctcagg tgtattcact gctgatcagct ttagtttaaact gtagatgtatg 2040
gataaatgtt atatatgctcag attacagactaatcagagagcattgaaa ttcggtgagaa 2100
gattcactata atagctgcatt gattgaaaaggg gttttctctgc tggataataat acaagatagaaa 2160
ttagattactgt ttgtagttact ttggtctgtgt atctcagagtt gtttattttct tggagttggttt 2220
gtttagttcctg tggaggtgatt tactggaatt aaatggtgataa tctggttattttct ttaggaggaag aaaaaaaaaa 2280
gacagtgtgg taaggacact atcattttact aatgtgataac gtttaataagaa tactagttgca 2340
acaatgtactg tagaagactgtt tggtaataaaa cctagtacag gtcttttgctgc tagtaggggtt 2400
gatagatagtgt ttttcttcctg gttgtggtata gttctgtatt ttagaataaa atagattttgtt 2460
gatattgata catctgtgca gttttataaa atcattttcct tttagaaataa aagaaatatactag gtagatttatt 2520
tgttttatag atatatattataa aaaaaaaaaa gaaatatatt ttagaactctgt tttaatatatt 2580
atggatttaa aaaaaaaaagacctgattc aatattttct gataaatataa aacaagctaa acaagaaaa 2640
atatccaggt atgtaatgga acaatctagt ggtgtttatg atgatgttat gtcacaaatg 2700
ccttatacag atgaaaaata tttatattaag tttttaaac atattattcc gttttttttc 2760
aaatatct ttaacatgga tcttatatatct tcaatggaat gaaattttagt agatgaatag 2820
ttccttatga gaaaggcagc cttacaagat aatgtgtatt tttttaaggat attttttttt 2880
atagtgttgtgc atgtgaaataa aactgcaggt acaataaaga aaattcagtc gtatctaaa 2940
cagtgtgatg aataacgaga aaagattaaa aagtgaatct taaggcaagg aaagaagaa 3000
agttgatgtg cgaaatttac agatcatttt agtgaaaaaa aggaagacct tttgttttta 3060
ttagataaaa taqaaaaaga actgaatatta actaagcaag tttaactaa acttataac 3120
gaaaaagagc ggtattataac aggagatttt gcattataaa gatatatttg atcagttatt 3180
gtttttgata gttggaatt tgatgataag gcgaacaggg ttggcaaaaa taataaaga 3240
ctagcaccat atggtgtagt ttaggaagaa aaaaataa tctaggtttt 3300
gtgaagtgta ttttgggtga gttccaggttt ttttataaa atatatgtgaaa aaaaatatttt 3360
atagttcaac aatactcatg aatacgcaca tctgaagttg tgtcagcccc caaaatattt 3420
gattgagaaa ttaggtgggt cttcctttat gatacttttag aaggttgttag ggtctttgta 3480
gctatgagat tagacaaaaa cagtaaatgt aatgcacatt aa 3522
actataagaa taaaatgaaga aagagagat gtttttgtaa gtgctagtgc tcaaaactgat 780
atgaaagta ataagggtag attatcgggt tcttagattag agaaaccaga tggaaaaagg 840
gatgtactct atacqacttg tacaggtagt actagggatat aactatatag gaaaaagata 900
gtacctgaag gttgatactat aagaataaat gaaaaaaaaga gatgtttttt tgtaagtgct 960
agttctcaaata cttggtgatag gaaaagttg cacattaata tatctggttc tagatttagag 1020
aaaccagagt agaaagggaacctgactg atgacaaggat acgaggtgtga caggtactac taaggataaa 1080
tctgttagag aggaaagtagt acctgaagggt gatactataaa gaaatataa gaaaaaagaga 1140
gatttttttg taagtgctag tgtcaaaact ggtgatagta aaagtgtca cattaaatata 1200
tctgtttctca gccataagagaa accagagtag agaaggagtg ttcgtgatac aggttgtacg 1260
ggttaataacta aggataaatc tgtgagggaa aagtagtatc clgagggtag tactataaga 1320
ataataagaa aaaaaggagag tgtttttgta agtgctagtg ctgataactggtg tgaatgaaa 1380
agttactcaag tttaaatttct tgtttcttaga ttagaataac tagatgagag aagaggtgt 1440
actgataacag gtgtgtaagg gttataacta ctgataaatct gtagagaaaa agtagttactt 1500
gaggtactgt ctaataagagaa tggaaaaagagag ttaggtgtgtg ctgaggtctac 1560	ttaatacttc aagttggttaa tgtaataagat gataagttaa aactactagtg ttagaatatta 1620
ggttaaatag aggattcagt ttatctgct cttagttgtg aaactactgta taaggttaaat 1680
aagcctgtta tatgtgttgg aaaaagaaagt acgtttcataat tagattcaag tttggatttg 1740
gttaatgctg ttgaaagata tgtcaagaatat aacctgtgatt ttaagttgaaac tgttctttta 1800
agttagatt tgtcagaaaat tggtaatctct gtagataagagggactag tagattaggt 1860
cctgatgctct atctgtgataa tgtttataat cactaataag aaaaacatttg tgggtgttggt 1920
cctcctcaaat cagaagatcata ctatttactca tgttagacta atgtgcatag tcaataatag 1980
gtgcactgaa aataagttaag tgtataacccg cgtgttattgt tgtcttgata tataaaacaa 2040
gctaaacagga atgaagatcac taacaggggt gcccctattag atacagtttca ccacccggcg 2100
gctgcgcata gcataaatgta tggactgaa aatagcgttaa gttgatgtaa gatgtgcaata 2160
gttcctgatg atataaacaag actaaacagag aatgaagata ctaaaacagg tggatttataa 2220
gctacaggtt ctacacacgcc ggctggccat agctaatatat agtgtactga aaatttgtct 2280
agttgatatact aagttgacctt agttcctgatg atataaaac aagctaaca aatggaagagat 2340
actaaccagg gtgtcttttat agctacaggt tcgcaacccg cggctggccaa tagctaatat 2400
gatagactgt aaaaataggt aagtttgatt cttgctctgatg tgtataaaa 2460
caagctaaac agaatgaaga tactaggcaggt ggtgctttta tagttacagg ttcgtgtatct 2520
gctaagtttag attttgttga tggtagtaat ttaggaga aaacgtgatat tgaatgaaaaa 2580
gttttaagt catcagttgg tactactgtct gattcagtaa gtaaatcctgt aggtatggaat 2640
aagtctcaat attgtgtaccc tggacttagag aggagagtga aatggtgact tggataaggat 2700
cactataata tggctagtat ggaaaaagtgt tataactgata gagaagttgt tgaacaatta 2760
agtaattgtta ctacttggtct ggttaagtgt ccagtaattg agcatagagt tcataagtgtt 2820
gagtctggttg cagagttgaca agtaaaataa ggtctctttag atgagggaaa atgtagagac 2880
agtgtgtgtaa tggagctcaat atttactagt gatacatgtt taaaagatac aggtgcaaca 2940
atgactgtag aagaatatgg taataacacct agtacaggtc ttttggtctag tagaggtgtat 3000
gatagtgttt tttctatgat tggataagtct tggataggttt ctgtatatttt tagataagat gattggttat 3060
atgtgtacta ctgtgcagcct taaataataca tttttctacct tagaaaaaaag aaaaacaattt 3120
atttatagtg statataaaaa aaataaatgaa aaaaatattt tagataat tgaactctgtg taataattatg 3180
gatatataaa aagaaacgtt aggatttacca aaaaaatttaatgttgtagataa cttaaaagtac agatgatata 3240
tccaggttag taaaatgaaca atctaggtggt tttttattag atgtatagtgc acaaatgtcct 3300
atcataagtg aaaaaatatttt attttaaggtc tttttctagct tagattttgtc tttttcctaa 3360
atatctctta acaattgaccc tataattttag aatggaatga aattagataa tgaattttctc 3420
tctatgagaa ggccagcttttt acaagataaat gttgtattttt taaag gtatgatatattgata 3480
gtgttgtgcat gtgaaaaaaac tgcagtgcgc ataacaagaa tttcagtcatt atctaaacag 3540
tgtgatgaaa taccgagaaa cattcagttgct gtaaatcctct ggcacaggaa gaagaaagttt 3600
gcattgtgca aacttacagga tcatttttagt gaaaaaagg aagacgtgtt tgtttattta 3660
gatataaaag aaaaaaatag gaatttaact aagcaagtttt acaactaatct tatagcagaa 3720
aagagggcgt tattaacagg agatgttgcct ttttttgattac agctttattgct 3780
ctttagattg gaaatattttgc tggataaggtct tggatagtttt cacaagttta tcaaaatata ataaagcctta 3840
gcaccataatgt tgttgtgtgtat agagagggaa aaaaaatattt aggttgggtttgct 3900
aagttgtatag tgttgtgtta ctttttgttag tataagagata taatatgttct tttttctgctg 3960
gttcagagagat tctataacgtt acgcacatct aagaatgtgctc ccgcggagaa atttttatatag 4020
cagagaaatgg atggttatattt ttttctacttag gatactttag aagatgtagg ggactttgtgta 4080
gctatgggaat tagcgacagaa cagtaaatgt atgcaacatt aat 4122

<210> 9
<211> 2856
<212> DNA
<213> Ehrlichia ruminantium
<400> 9
ttgcataaaa tcattgtttc atcaactttaa actacagttta ctgataatatt acaagcttct
60
tagattaat atggcgcttt gtttatatttt tttttaaagg gcaacattat tttttctgta
120
tattatacaaa gaaataaatgt taattgatat gatgcaaatatt gtagatcttt acaatgggct
180
ttttattttt attgatgataa atccttatttg atgttggcac aacaatttatt
240
cacaccaga gttgatttttt aagaaataatgt aatgatattt gatgcaaatatt tggattttctc
300
gaaagaagt tatacgtagaa agctttaggtt atcaaatatatt ataatatttatt ataatatttttcaaa
360
tatgtgcttt taccaaatatt acaatgcatgtaa caaatttatt tggatgatcatt gttgtgtatt
420
gcaagaagttg gaatactctatt cttattttac tggataacgg gtggtagatt tattgttgtatt
480
ttcgatgagtag taatatgacta tcgcgtgtatt tttttggttga tggatgcgct
540
ttgatatac ataatataag atttgaaatt tatttttaaa ataatgggtgtagcagatttt
600
aatgtgtgcct attaccattaa gcattctcgttt tatattatatt tattttaggtt atcaagtattga
660
taatgtgtaa aattagagatg gtaagcgtttt ataaatgtttt gaaaccattt
720
tctaggatt acaatatttttt tttcagtatta gttgattttt gtagtatttata tattgattatcat
780
tctcatcag aagaatttttttgtgggttttt tttttcatttt gtaaatgcag taggtggtttg
840
aatatatata tttttatcatag tttatatatt gttatttataa ttgatattttttt cagcaaatattttt
900
gtataatag catatatggg tattgttattt gttatttttatttt gtagggttttattgtttttttt
960
tttatagt aatataggg gatgattattt gttattttttt gtaattgttttt gttatttttttattttttattttt
1020
gaatatgtaa tttttttttttttttttttttt gttattttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
tcaatactga ctaatagag cgtaggtgta ttttaaagta cagctagtcg tattaatctat 1920
gcttttggtt atgtaataaa gcctactcgc catgtaacgc atgtacatt gcaaccgaag 1980
tcaccatatg gttaagaggt tgtgagccat ataaatccta aaacggataa ttctatacat 2040
acaagtcga tccaagatgc agtcctgact agttaaggct atgtagttt taaaagttcg 2100
gctagtccta ttaatccatg ttttggttat atgaaacctg ctatgtctgt tggtagtgcga 2160	ttagttgata ctgatgttcc aaagcaagtg gaaagttgta gtatggtccc agtacatctt 2220
tctctacag taaagtcggt attagtaggt gatggtatgc atgtatctgg cagtgaaaaa 2280
gatagttggt gacatgaaca agatgggtgt catggtgatg ttagactag tcttttattg 2340
aataaatga atgtaaattg atccaccaat attaggagcc atgtgaattc ttcttttagct 2400
ataaaacata agatattagg tagaagaagta aagtataata taagggctag tctgtttaga 2460
tctgggtgtta atatcgcgca taaaaggtca tggagctaca gatataacaac tacagccgata 2520
caagaggctg ataataatgg tggtaacctg ttaatccatg cacagctcag tattaggatt 2580
tataattgta gttattttaa tagtaattct gottttattg atggaagttc tggatttagg 2640
aagaagtga tttgcgctat attttagtata ttcgtttact ttattttatt agttggactt 2700
aataattgata agttggtattt acgaagtttg aatagaagaa gctatgtctg tagaatacag 2760
ggatgttggg tttataattt gatagttgatt tttctggtttgt gcagtgacag 2820
aaggttcca catctgcaat agagatgcct tagcttgatc 2856

<210> 10
<211> 1659
<212> DNA
<213> Ehrlichia ruminantium

<240> 10
atgttaccca aacccgttct acacccattac cagatttaca cattcccttgca tagatcagtt 60
atatcggtggt tgaaagagtc gtcgagaataa catgtaataa cggtgtaatt cattaggact 120
acaataata aaaaaacttc taatatcttg ccgttataata agccaggtaa tgggtgtact 180
cacagtggga catcctttact ccgttaggaa ttataattgc aagttctcttg aataaatataa 240
cacgtagata tatacttaca aagattgtca caagttttgt ctatgcttttt ttaataaca 300
catgatccac ttaaggggtg tgcgttattt atttgatttc aaggaagatt aaggaatattc 360
ataagatttc taataactttt atctagttac ccagctgaag gactttttac tatattatat 420
attgtataat cctggaactt ttctttattg agggcagaaaa gtatgggtaa tagagatgta 480
agggatagtt ccctttttaa cgaatccag gcctttaata ttttgagggt tctatgaggat 540
tcttttttt ttttaaacc ccctataacat cctgatata cgcacgcga aacacatgtaa tacaaacaa 600
catagcag atagtaaca acaaaaagt gtagtaactg gtaaggaat tcaacacaa

acacaacata taaacaaat gtttctcaga gtttctcact cacaaggttc accttcaagt

lactacsgtc gctgctcaat gacacataat atagaagaa aacacgcaag tagtccgcaa

dagcaacgtag tcaasaggt tagatgaact agcaaaacac ctcgtaaaga agaaataaca

attatatata caaagtttcc tgacgctca gaagaacctt catcttcaaa tcaacacaa

gagacaacaa gaagcatttt aagcaagcag ctaatccaaa gtagatagg tgggtgcggca

tttatcaaca aaggtcagtt aagctccaca caagttgcc gacccaaacct atttgcctct

agtccttttt ataaagaact gcacccctca atctaaatta aatccaaaca

cagaaacac agacacga ctttagaacag ctcctcatca aaaaactaca aaaaaggtaca

ataaccccttc aacaagttgt tagaaccaaa acacaatttt catctcttcc tttttataaa

aagacgcgtag gtagttttag attaaaaaaa agtgacactag gtagatgcc gcctatctata

caaaaaggtta caataatcc tcaaaaggtt gtaggcacaa aacacacact tcgatcagtt

cccttttaca aagatgaagac ccttcacaac ataaacactc aatagttcagacctatgaa

aratccgcaaa atagttgtgt accaatattat acaattagtg ccctttttaa accaaggtttt

tccttggtag attatttttag tagttataaa gtagttgacg tatacttaca agaactttat

aaaagttgata ctttacccac agcacccttc gttccctcat gtaaccccga agatgatgta

tttgttgaaa atagtaatcc ccatgttatct ttaaatttaa

<210> 11
<211> 873
<212> DNA
<213> Ehrlichia ruminantium

<400> 11
agcacaacctt ctttgcagct atatggcata gaaaaagat taaatcttct tctatgcaat 60

gttataaatt cccgtgtaaa tgtgatgcaaa gaaatagata tatctttgtg aggatatcctt 120

gatgaacccg gtaaattagt gcttttttatt gacccctaaca tttcctcataa cttatatcctt 180

attctccaa tattaaccctt atagttgata atgtgctcta tataaaccat ctaattacc 240

aatctcattt catcaagatt atctaaagag tttgttttca cagaaggtta aataaatagt 300

ggcctttgta agttttggaat gttttttttt gtagttgaag aaaaattcgg tagttttta 360

gaaatagta gtaaccccctaa aatagttgta gaaaaatttta taaaaccttt ctaaatattt 420

tcataacaact tattttcaga agatatattta aatccctcta ttaaacataa acatgcctca 480

tcaaatatat ccaaaattaa tgcagaaatg atatgtgcct cgtgtctagc aagaattga 540
aaacttaagc agcttaaaga aagaaatgat gatattatgta atggaatatac ccattgcaggt 600
aatgaggaaga atgtacttac tgtaaatatc agtagcacat ctctcagacat taaaactatac 660
acagttcata ttaaagtgcc agaaagctct ttatccattac cttcaaatgta ctctctatattt 720
ccttcagaga tgatagctat ccctaatgtt atagggggca atcaaatatt actctctact 780
ttgaattaa cagaaagtga atgttaaaaa ggtgctctcta atgtgcaaat gtaacctttta 840
gttagaagg agaaactttaa aaaaaattttac tga 873

<210> 12
<211> 1740
<212> DNA
<213> Ehrlichia ruminantium

<400> 12
atggctatac cacacactat tatagcaaat aatggcctat tatctttctac tttaaagta 60
ataaaaatgt actcttggtat tgaatgtagt tatatttttat gcactgcata ctgctgtaggta 120
actaagcata acctctcaaga ttatcctaat gaagtgctct cggatgatgt aatagatgat 180
actacacacac aaaaaagctca aaaaatttaa aatatacatta aatttgcttc tgtaaatagttg 240
gttaacctctc atacacaaaga aatgttatca ttaaactttaa gtgaagaaga actatxacaac 300
gataaggtgct atagtaaatgc aacatttcaaaa tatttaagta atctgctcaca agaaagatt 360
gataacttta gacaagaana taataaatata tattgtgaaat tgtctttaaa actataataaa 420
catcaacacaa acaataaaatg aatagccatat agtatcttct gtaaatgtct gttaaagttc 480
actcaagtct caagagatct cttctcttttt caataaaatc aattttttta cttataagg 540
atataataac caccttaatac tatattcatacg atggaaaact ttaataatac aattaaataa 600
aaaaaaagtg aaaaaagaaa tttttaatacg atattgctata tgttattgct agtatagtgcgaa 660
gagatattga agatattttac tgtgtgtgct gatggattgta atagatctgatt attaaaac 720
ttaatttcaac attttttcgt aaaaaagttta aattttatcta atcttmatac cacaagataa 780
gatatagtgta aaaaagaaaattt aatacaatcata aatgggttat gtaaatgagta gttttat 840
gttgaagata gacagataat tgaatcagta ctagcacaacac atggatatac cgaataaacaa 900
gcaaaaacac tootattgcaag aatattttgct acacttttcttt ctttatataaa aaaaagattttt 960
cctcaaaaag atagcttgcttt tatagtaaatc aatgtatagc cttgacaaggt ccaagagcttctttttc atctttcttttt 1080
ttaataacgaa gtagattgtt aacattaaagtt atatttaatc tctctcgaatc agtggatagta 1140
cataatcttaaa gtagataaga aatgtcgagt aagattttttattagtttat atatggtcat atttcgctta 1200
gtagataatc aaaaacatatt tcttatataag aatttttgta ccaagaacac cagtttttttct 1260
gatgttgtct ttagcagatt atcagatgat atatctatgcta atacaatagata attataataata ctataatcata ctaatcttatgctaatattcatt 1320

<210> 13
<211> 3252
<212> DNA
<213> Ehrlichia ruminantiurn

<400> 13
ttgatataata atataatcata atcataaatcata atataataata atataataataata atataataataata 60

<200> 13
ttgatataata atataatcata atcataaatcata atataataataata atataataataata 60

<210> 13
<211> 3252
<212> DNA
<213> Ehrlichia ruminantiurn

<400> 13
gatcatgttg atgttataaa aattcaoacgt aagttagtga aaatattgc taatatttaca
1200
tatgttggtg atacaatttt aggttttgat aagtggtaac atatattatg tcgtaatgtg
1260
ttagctcaaa aatcaactgt taattataag gtatctcag ttttttttaa ttagagaa
1320
atgggaatttg gtgtattatt taagagttgg agtggtgaaat ataatgactt gttaataggt
1380	
tataatagtc ctgtcaggtta tggtgtaataa tatactcag aatatttaag cgatgttata
1440
actgtttcag gtastgcaggg tttttagtagag aagttctaat caactgtaa aatgggtgat
1500
gtatttaaga ttacagataa ctttaattaat tatactagt gaaacacctac taacatagt
1560
gcacatagta cattgcaact aaaaaaagtc gatggtgagg gtattacaga gcgtgactgt
1620
aataaatcg ataatcttgt aatgtgaagg ttagctacag gattttgtct tactagtaaa
1680
aatagagtat tgtttaaaag tcaagctagtt ccatattaac atgccttttg ggattgtaata
1740
aagccactec gccagtaaac gcatgtaaca ttggaatcga agtacacctta tggtaaagcgt
1800
gttgtagaac atatatgtcc taaaagtgaat aatcttataac atacaatggt gatctacaag
1860
tcagtactga ctagagagc cgtatggtta ctagaatcga tagaactatc tattataactac
1920
gctttttgtt aatgaataaa gcctactcgc catgtaacgc atgtaatcatt ggaatcagag
1980
ttaccatag taataaggtgt ttagtcgctta atgtaacttct aacagcttaaa ttctatatag
2040
acaagcttca taccaagact aggtaaaggc atgtgtatatt gaaagtaacatca
2100
gctagtccta ttaatcatgc ttttgtttatat gtataaaagc tcatcgcctca tgaacgcgt
2160
taatccattg gatacagat ttcatacata ataaaggttg tgaagccatg gatccctaca
2220
aagggataact tatacataac aagtgcaata ccaagatcag tattagactag tagaagccgt
2280
gatgtattgaa aagttcagtc tagtctctatt ccatgatgtt tgggttatgt attaaagccct
2340
actcactatag taacagctagtt aacatgggaac tccaggtcct ccatgtttaa agaggttggtg
2400
agggcataatg atcctaaacc ggtaaacttc atacatcaca gtctcaatacc aagatcaagta
2460
tcgactagta aacgctagta tgtattttaaa aagttcagcta gcctatattta tgaatgtttt
2520
ggttatatga aacgtctagtt tctatatgta gatcattatg atgtagctga tgtcttcaabg
2580
caatggaaa tggtagttga tggcctagta tattttcttcctacgtaga atcagtgatt tctataagta
2640
gatggtcattt gctgctatgt aatctggagt gaaaagata gattaggcata gtaacagatgt
2700
ttgggtcatg gtgtgttttag gccagtttctg tagttgataa ctaattggtga taattgtagc
2760
aacattata gtagcctagt aaatgatctct ttagctaataa aacataagat aatagtatgaa
2820
aatataaagg ataatataag gcctagtact ctgtagcttg ctgtaaatat tcgcaataaa
2880
agtacagtga gtacaagata tacacatcct ggcatacaag aacctaatata ttgatgtgtt
2940
acatttgtta atcctacaca gcataaatatt agtagttata atggtagttt attaatagtt 3000
aattctgcttt ttaatactga aagttctgtt gattataaat tagtaattgc agtatattct 3060
agatatctgc ttatcttttt attatatgg gtatatataat gtaaaagtct gtatttagca 3120
aagttgata ggaagaaggat gtctataaat gaaaggaggt ttgtgatatt taattatttgat 3180
agattccaaa tgtgtggggt tgggtggcga gtgacagaag gtaccaacatc tcaaatagag 3240
agcttattctt ag 3252

<210> 14
<211> 1836
<212> DNA
<213> Ehrlichia ruminantium

<400> 14
atgtttatcc aacccgggttc accggtgcc cagattacag aatctctgca taaatcagtt 60
atatatgagt tgaataagagt accgaagaata catccttaata catgtcaatt gatagagct 120
acaattagta caaaaactgga tatctggtatt gataataagtt caggtcatcg gtgtactcca 180
gtaggaacat ctttatattt ctggaatgtt attatccca ctggtgtaat aaattcatca 240
cgaatatat tccaattcaaa gttgacacaa gtaagttgct gtcggtttttc aagcaacca 300
ccacctagg cttatgtata tttattgtca tcaggaaggg aacctgaaag ttcaginaagt 360
acagtagttc ctttattgaa ttagggagtt ttaaggagtc tagaggattc taaatgtgta 420
aatcggag ccatttgcgt atgtaggcca aaaagtatag gtaataaggg ttagatgtta 480
cattttgtg tatacaagac ttttaagggtt ttaggaggtc tagaggattc taaatgttta 540
saacacccc ttatcaaccc ccatacacc aacgtaata caaaaaagg cacagcaaat 600
aatgagagac aaaaaattgtg atgaactgtg tggaaattc aaagcaaaat aacaaggtata 660
aaaccttac ataaatatatt ttcctagatt ttcattcacc aatgtcacc ttaagctca 720
ccaattca tccaacacaa acataataa ggaagaaac gccaaaggtc tagcaacaaa 780
ccaaatattc caaaaaggtttc agtaacttgt aatacaacct ccagcaaaa ctgagaaactc 840
atatgtcaaa gggtccgctt aatcctcactc taaatcagag ccacagagaga 900
gagagcaagt attagagac cggcttagat caaaaaagag tagatagtag agatcagaaaaact 960
caaaagtta caacagattc tcaacaagtt gtagacagaa aacacaatt tggcattcag 1020
cctctttat ttagataaga accgccactt ttaactcaac AACATCTCACAAAATTCAA 1080
agacacaaga caggagggaa gtaatagga cagcgccctc caaaaagtagt agcagagat 1140
acgccatttt cccaagaaagg tcaacagat ttcacacagg tggtagaacg aaaaacaca 1200
ttaggatca gttacttttttt ttatatatc aaacagagaa tttatgactc aaacatcctca 1260
tcttcaatct aagacacaag gacaggaggc aagtatttag aacagcgcct atcaaaaagt 1320
acagcagata gtacgcctatt tacacaaatg ggtacaacac atcctcaaca agttgtttaga 1380
ccaaaaacat ttgcatctcg tcctttttat aaagaatcgg cactgcaatt tacaacacac 1440
cocatctcaaa atcaagacaag aagcccaaca aacacgcatt tgsacacagc tctcatcaaca 1500
aaaagtacag tagatccctca acaagtttgtt agggccaaaaa cacaacccgc atctagctgt 1560
agttataag aagagggact tcaacactaca aacacataaa tattaagtgc tataaagaa 1620
tctcctgata gtacgtacac atggaaatca ttgaagtctg aagaagattt acggtttttta 1680
aatgatagatt atctagtag ttgtgaaata ttatatcagata cttccagaga atcttataga 1740
gttagtgcct tacaacactc acctctcctc cccatcactc aacctgaagc tgtgatattt 1800
gttgaagatg tgtatctctcg tgcatactat cttttg 1836

<210> 15
<211> 3570
<212> DNA
<213> Ehrlichia ruminantium

<400> 15
atgccactta cttttgatct atatgcatat gaaagaaaat taaattttct tctatgcaat 60
gctgttaaatg ctaatctcaa gttaagtttta ttaataaatg tagtttgtgt aggatatact 120
gatgaaata atcagttatt actgtgtacat gactacaaca tttcaccaga attacacccct 180
atctctagaa atcaatcctt atttcgtata aatgctataa taaaacctag ctttataaca 240
aattttttta gattatctca aagttttgtgct ttaacaccaag aggacataga taatttgtaat 300
gttaagttggt aatgtggttg tttagtttgtt aatgaaatac ttgtgatttt tactaaata 360
tgtgtagatg attaagtaag atataaaat ctaacaacaa ttttcaagaa tatttacaca 420
caatcttta cagcgagatt atttaaaaat cctattaaca tagacatgc tctatcaata 480
atagcacaatt taatgcacaca atatatatg gcacgtagtc taataatga atctgatcata 540
ataaatgctt ctgactctta aaatgcgcct gttcaaataa aagagaaaaa atataagat 600
attagaggaat gtaagcttag attatatgat gcaatagtcg gtaaaatgc gaactatgtg 660
taccatgtac ttctgttaaa atgcataaggata atactctata ataatcagca actcatagtt 720
cacactcaaat gttccagaga cctttttacct atacaattca gtaactctct atttatggta 780
tgtgtgatatactcattcataa atataatggaa attatcctct atctttttgaa 840
ttacgagaa atgaaagtttaa aaaaaagtttaa atcaattttg atggatttaa tttagttaat 900
gatgaaacac tgtgaagtttta atctctataa tgaatataa atcaaatggcctt 960
cagatatta ttcnaatttgg ttcgtgataat tgtataacac tcaatacaga aagaatatca 1020
tcattacaa ttgctgaaga agagctaata aatagtgtag gaataggta tgtaacactc
agaatcttta gtgtctacag taaggaanaa tttaagaaaa tacaagcata aagaatgaa
catagttagt caaatgtcag tatatatgca gctaataact accatgtact tcgtgtaaa
agaaatttta gtgatctacg taaggaaaaa cttaagaaaa tacactaagt tccagacagc
tttttaccta taactccaaag taactctcta tttatgtaaa atgtgtgagt atccaccagat
atcataaca attaataaaaa attccctctct actctttgcatt taacagaaag tgaagatgaag
caaagtactc tcaagtgtgc aatgtactgt tgtatttaatg tgaataaact tgaagttttt
actataaat ggtatattac aataataaaca caaggcttc aagatattat tacattttgt
ctcgttaaatt gtataacact caatacagaa agaatggttat cattacaat tagcgaagaa
gagctaataa atagtgtgga aataggtgat gtaaatggataa aaaaatttag tgtatctactg
coggaaaaaat ttaataaat aacgcaaaaaa aataatgaac tgtgtgtgc aatatgcatt
tcactgaag aaaaataaat aatgtatata aatatgcag acacactagcgtaaagat
aaattagtga ttcatactga atgtcctcta gtcctttcttc ttcacactca agtgtttacta
atatattccta aacatgtttta aatgtagttt ggtattaata gtaatttata ttttacact
ucatagtgtc tagtatcaaa gcatagtctt caagatattta caatagaggt gtctcgagat
ggtgcaatag gtgatagtat acaacaaaaa cgtcacaatttt ttgaagattat cattaatta
tgttcctgtaa aatgtcttaac attacataca caagaatccgtgcatattaa atttagtccaa
aaagaactaa tcaacgtatat aggttatagt aatgcacatt tcaaatattt aagtaatctgt
cataagaaaa aatgtatctt ccataaacaag ttaaattata aattatgtcg tgaataatgt
aataaactta ggaacatata aacacaatatt atagatgtga taggaatctc tgttaatcct
aaattagtag ttccaactca ggttcactca gatcatcttc ttttuctaa aggtcactct
atatcaatta taagagatata tatatacaac taaacattatat acacagataaa aacactacgt
aatacattta aattaacaac aatggaagaac tccagatctac acataaattt gtagatgtat
tgcctagatgt atgaagaaaa tataagagtt ttatttgtag tattgtgatg tccaataatg
ccaatattgg aagagttatat ttaaatttgt tctgtttaaat gtataaatataa gcatacaca
aataatgttat cattaaacat tagtggaaga caactaatcag acgatataag gtatgtataat
gcagaattta aatagtgtgga aagtaacata ataatagatt cattattgga cgaatttaaat
tataatgaaa tattttctca aacaagagata gttgtatag tgaatatattt aatttcggtga
caagatattta caatattatgc tttctgattata tcaccaagagg aattttatcc caacttcaga
ggtacatcct tatattaatt acgaactagg atattaactg aagttatat t aagtacct 2940

<210> 15
<211> DNA
<213> Ehrlichia ruminantium
<400> 16
atgagtcaca gttttattg a g 21

<210> 17
<211> DNA
<213> Ehrlichia ruminantium
<400> 17
cactcaaaat cacaagaagt a 21

<210> 18
<211> DNA
<213> Ehrlichia ruminantium
<400> 18
atgatattagt tctatattag agctg 25

<210> 19
<211> DNA
<213> Ehrlichia ruminantium
<400> 19
ataacatcct attggaacat ac 23
<210> 20
<211> 20
<212> DNA
<213> Ehrlichia ruminantium

<400> 20
atgaaaggat ctttatctgc

<210> 21
<211> 20
<212> DNA
<213> Ehrlichia ruminantium

<400> 21
ctttctttt cttcattatg

<210> 22
<211> 19
<212> DNA
<213> Ehrlichia ruminantium

<400> 22
aagaattaca tgatgcagc

<210> 23
<211> 22
<212> DNA
<213> Ehrlichia ruminantium

<400> 23
tcttctttg ttatacttc tg

<210> 24
<211> 23
<212> DNA
<213> Ehrlichia ruminantium

<400> 24
atggatttaa atasactsat aaa

<210> 25
<211> 19
<212> DNA
<213> Ehrlichia ruminantium

<400> 25
gcattttctc tacctacga

<210> 26
<211> 26
<212> DNA
<213> Ehrlichia ruminantium

<400> 26
gtacatagta tgtttttata taaaag 26

<210> 27
<211> 23
<212> DNA
<213> Ehrlichia ruminantium

<400> 27
ccaaataat aatgtctct ttc 23

<210> 28
<211> 25
<212> DNA
<213> Ehrlichia ruminantium

<400> 28
tccaccagag atgttatttg taaag 25

<210> 29
<211> 25
<212> DNA
<213> Ehrlichia ruminantium

<400> 29
cacagaa act ttcgtattt aaagc 25

<210> 30
<211> 25
<212> DNA
<213> Ehrlichia ruminantium

<400> 30
gttaagtgtg aatgtattg tttag 25

<210> 31
<211> 25
<212> DNA
<213> Ehrlichia ruminantium

<400> 31
cactttctgt taattcaaaa gtga 25

<210> 32
<211> 25
<212> DNA
<213> Ehrlichia ruminantium

<400> 32
gtaggccaa aagtataggt aatag 25

<210> 33
<211> 25
<212> DNA
<213> Ehrlichia ruminantium
<400> 33
caacacaatac atcatcttcg agttg 25

<210> 34
<211> 25
<212> DNA
<213> Ehrlichia ruminantium

<400> 34
agggtactt attgtagtcagagtg 25

<210> 35
<211> 25
<212> DNA
<213> Ehrlichia ruminantium

<400> 35
cctttcgta tacaggatta ccatt 25
A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N1/20 C12N15/09 C12Q1/68

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)

C12Q

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 practical, search terms used)

EPO-Internal, EMBASE, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>WO 2006/045338 A (AGRonomIque pour le DEV CIRAD [FR]; CENTRE NAT RECH SCIENT [FR]; FRUTO) 4 May 2006 (2006-05-04) cited in the application page 2, line 19 - page 14, line 28; sequences 1-6, 9, 11, 13, 20</td>
<td>1-3</td>
</tr>
<tr>
<td>A</td>
<td>FRUTOS R ET AL: &quot;Comparative genomic analysis of three strains of Ehrlichia ruminantium reveals an active process of genome size plasticity&quot; JOURNAL OF BACTERIOLOGY 2006 UNITED STATES, vol. 188, no. 7, April 2006 (2006-04), pages 2533-2542, XP002430891 ISSN: 0021-9193 cited in the application the whole document</td>
<td>1-3</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. See patent family annex. 

* Special categories of cited documents:

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

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, talk, exhibition or other means

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

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered noval or cannot be considered to involve an inventive step when the document is taken alone

'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.

'Z' document member of the same patent family

Date of the actual completion of the international search

24 April 2007

Date of mailing of the international search report

09/05/2007

Name and mailing address of the ISA/Authorized officer

European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 940-2040, Tx. 31 651 epo nl, Fax: (+31-70) 940-3016

Donath, Cornelia
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>ALLSOPP M T ET AL: &quot;Ehrlichia ruminantium major antigenic protein gene (mapl) variants are not geographically constrained and show no evidence of having evolved under positive selection pressure&quot; JOURNAL OF CLINICAL MICROBIOLOGY, WASHINGTON, DC, US, vol. 39, no. 11, November 2001 (2001-11), pages 4200-4203, XP002321870 ISSN: 0095-1137 the whole document</td>
<td>1-3</td>
</tr>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>WO 2006045338 A</td>
<td>04-05-2006</td>
<td>NONE</td>
</tr>
</tbody>
</table>