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(71) Applicant *(for all designated States except US)*: **INSTITUT PASTEUR** [FR/FR]; 25-28, rue du Docteur Roux, F-75724 Paris Cedex 15 (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **GUISO-MACLOUF, Nicole** [FR/FR]; 4, rue Christophe Colomb, F-75008 Paris (FR). **BOURSAUX-EUDE, Caroline** [FR/FR]; 9, rue du Colonel Fabien, F-92160 Antony (FR).

(74) Agents: **DESAIX, Anne** et al.; Ernest Gutmann-Yves Plasseraud S.A., 3, rue Chauveau-Lagarde, F-75008 Paris (FR).

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(54) Title: POLYPEPTIDES CONTAINING POLYMORPHISMS OF THE REPEATED REGIONS OF PERTACTIN IN *BORDETELLA PERTUSSIS, BORDETELLA PARAPERTUSSIS*, AND *BORDETELLA BRONCHISEPTICA*, THEIR USE IN DIAGNOSTICS, AND IN IMMUNOGENIC COMPOSITIONS

(57) Abstract: Pertactin (PRN) is an outer membrane protein expressed by *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica*, which induces protective immunity to *Bordetella* infections. The immunodominant and immunoprotective epitopes of pertactin include two repeated regions, I and II. Comparison of these two repeated regions showed the pertactin of *B. parapertussis*) is invariant, whereas the pertactin of *B. pertussis* varies mostly in region I and *B. bronchiseptica* varies in both the repeated regions I and II. Compositions containing pertactins and pertactin fragments containing variant sequences in these regions are useful as immunogenic compositions.

POLYPEPTIDES CONTAINING POLYMORPHISMS OF THE REPEATED REGIONS OF PERTACTIN IN *BORDETELLA PERTUSSIS, BORDETELLA PARAPERTUSSIS,* AND *BORDETELLA BRONCHISEPTICA*, THEIR USE IN DIAGNOSTICS, AND IN IMMUNOGENIC COMPOSITIONS

BACKGROUND OF THE INVENTION

This invention relates to proteins and polypeptides of the *Bordetella* outer membrane protein called pertactin and the polynucleotides that encode them. This invention also relates to the use of these proteins and polypeptides in immunogenic compositions, diagnostic methods, and diagnostic kits.

The genus *Bordetella* includes seven species. The most studied species are *B. pertussis, B. parapertussis*, and *B. bronchiseptica. B. pertussis* is responsible for respiratory infections only in humans. *B. parapertussis* causes infections in humans and sheep, and *B. bronchiseptica* infects many animal species, including humans.

These pathogens produce an array of virulence factors, the synthesis of which is regulated by the two-component, *bvg* AS (2, 21) system. These factors include toxins, such as pertussis toxin, which is the only toxin specific to *B. pertussis*, tracheal cytotoxin, adenylate cyclase-hemolysin, and adhesins, such as filamentous hemagglutinin, fimbriae, and pertactin (PRN).

PRN is an outer membrane protein with an apparent molecular weight of 69 kDa in *B. pertussis,* 70 kDa in *B. parapertussis*, and 68 kDa in *B. bronchiseptica* (5, 14, 15). The precursors of PRN are 91.5 kDa, 93 kDa, and 92.5 kDa in size, respectively. In *B. pertussis*, PRN has been demonstrated to be an agglutinogen (4), promoting attachment to certain eukaryotic cells via an Arg-Gly-Asp (RGD) motif (13).

Antibodies specific for the *B. bronchiseptica* PRN are detected at high titer in immunized piglets, whereas few if any of these antibodies are detected in unprotected animals (19). Synthesis of the PRN by *B. bronchiseptica* correlates with protection (16). The immunization of mice or piglets with preparations of the PRN induces protective immunity against *B. bronchiseptica* infection (12, 19) and passively administered monoclonal antibodies prevent the death of animals challenged with *B. bronchiseptica*

(16). *B. pertussis* PRN has also been shown to induce protective immunity to intracerebral, aerosol and intranasal challenges with *B. pertussis* in mice (11, 18, 20).

PRN is, therefore, now included in some acellular pertussis vaccines (i.e. vaccines composed of purified bacterial proteins) (9). However, the PRN proteins of these three species, although clearly related, have different immunogenic properties. For example, preparations of *B. pertussis* PRN protect mice against intranasal *B. pertussis* challenge but not against intranasal *B. parapertussis* challenge (11). They also protect mice against intracerebral *B. pertussis* challenge, whereas the *B. bronchiseptica* PRN protein does not (18).

Comparison of the deduced amino acids of the three proteins, *B. pertussis*-PRN, *B. parapertussis*-PRN, and *B. bronchiseptica*-PRN, reveals a high degree of similarity, with the *B. bronchiseptica* and *B. parapertussis* proteins being more similar to each other than to the *B. pertussis* PRN protein (5, 14, 15).

The sequences of the three proteins differ in the number of repeats in regions I and II (Fig. 1a). Using monoclonal antibodies, Charles *et al.*, identified and characterized a protective immunodominant epitope of the P.69-PRN protein (6). This epitope spans the (Pro-Gln-Pro)5 repeat sequences located in region II. Differences in this region may account for the observation that sera from piglets that recognize *B. bronchiseptica* PRN do not react with *B. pertussis* PRN despite the high degree of similarity between these proteins (12) and for the lack of cross protection provided by the three proteins (11, 18, 20).

It has recently been shown that the PRN produced by clinical isolates of *B. pertussis* varies. Sequences of the *prn* gene of various clinical isolates revealed three major types of PRN variant (17). It has been suggested that epidemics in the Netherlands result from changes in the sequences of the genes encoding PRN and PT because the proteins present in the clinical isolates currently in circulation differ in sequence from those observed by the vaccinal strains used in this country (17).

For PRN of *B. pertussis*, all the observed amino acid differences are located in region I. The allelic *prn* types A=1 and C=3 are very similar, differing by only two amino acids, whereas type B=2 is quite different, having a five-amino acid insertion in the same region (17).

Only one type was found to differ in region II. This type (A*=6) is produced by the *B. pertussis* WHO reference strain 18323 and one French clinical isolate (3). It does not, however, seem to be common because it has been detected in only one clinical isolate (3). The production by this *B. pertussis* strain of this unusual type of PRN reflects the many common properties shared with the *B. parapertussis* and *B. bronchiseptica* species. No differences were found in the phenotype and behavior in the animal model of *B. pertussis* clinical isolates with different PRN (3).

There is a need in the art for compositions containing proteins and polypeptides of *Bordetella* pertactins that can be used in immunogenic compositions to protect against *Bordetella* infection and to treat subjects infected with *Bordetella*. Ideally, the proteins, polypeptides, and the polynucleotides that encode them would also be useful in diagnosing *Bordetella* infection and in kits for the diagnosis of such infection.

SUMMARY OF THE INVENTION

This invention aids in fulfilling these needs in the art. In one embodiment, this invention provides an immunogenic composition comprising a mixture of pertactins of *Bordetella* species, wherein said composition comprises: (a) pertactin of *Bordetella parapertussis*, and (b) pertactin of *Bordetella bronchiseptica*, in amounts sufficient to induce a humoral or cellular immune response against *Bordetella parapertussis* and *Bordetella bronchiseptica* in an animal to which the immunogenic composition is administered. The immunogenic composition can also comprise pertactin of *Bordetella pertussis* in an amount sufficient to induce a humoral or cellular immune response against *Bordetella pertussis* in an animal to which the immunogenic composition is administered.

In another embodiment, the immunogenic composition of the invention comprises a mixture of pertactins of *Bordetella* species or fragments thereof. Specifically, the mixture comprises a mixture of *Bordetella bronchiseptica* pertactin variants wherein each *Bordetella bronchiseptica* pertactin variant comprises 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof. The *Bordetella bronchiseptica* pertactin variants are present in amounts sufficient to induce a humoral or cellular immune response against *Bordetella bronchiseptica* in an animal to which the immunogenic composition is administered. This immunogenic composition can also comprise pertactins of *Bordetella*

parapertussis, *Bordetella pertussis*, or mixtures thereof, in amounts sufficient to induce a humoral or cellular immune response against *Bordetella parapertussis* or *Bordetella pertussis* in an animal to which the immunogenic composition is administered.

In a further embodiment of the invention, the immunogenic composition comprises a mixture of pertactins of *Bordetella* species or fragments thereof, wherein mixture comprises a mixture of *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof. The *Bordetella bronchiseptica* pertactin variants are present in amounts sufficient to induce a humoral or cellular immune response against *Bordetella bronchiseptica* in an animal to which the immunogenic composition is administered. This immunogenic composition can also comprise pertactins of *Bordetella parapertussis*, *Bordetella pertussis*, or mixtures thereof, in amounts sufficient to induce a humoral or cellular immune response against *Bordetella parapertussis* or *Bordetella pertussis* in an animal to which the immunogenic composition is administered.

The compositions of the invention can comprise a mixture of fragments of the pertactins of *Bordetella* species. The immunogenic compositions can also comprise at least one polypeptide of the invention in an amount sufficient to induce an immunogenic or protective response *in vivo*, and a pharmaceutically acceptable carrier therefor. In addition, the immunogenic composition can comprise a neutralizing amount of at least one polypeptide of the invention.

A preferred immunogenic composition of this invention comprises a mixture of pertactins of *Bordetella bronchiseptica* species or fragments thereof, wherein the pertactins or fragments thereof comprise a mixture of *Bordetella bronchiseptica* pertactin variants in which at least one of the *Bordetella bronchiseptica* pertactin variants comprises Region II of pertactin of *Bordetella bronchiseptica* having 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and at least another of the *Bordetella bronchiseptica* pertactin variants comprises Region I of pertactin of *Bordetella bronchiseptica* having 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof.

In another preferred embodiment, the immunogenic composition of the invention consists essentially of (A) a polypeptide comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin

of *Bordetella pertussis*; (B) a polypeptide comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin of *Bordetella parapertussis*; (C) a polypeptide comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin of *Bordetella bronchiseptica* strain 9.73 and a polypeptide comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin of *Bordetella bronchiseptica* of strain SEI.

This invention also provides polynucleotides encoding the proteins and polypeptides of the invention, as well as antibodies that recognize the proteins and polypeptides. Also provided is a DNA chip, wherein said chip comprises at least one polynucleotide according to the invention or fragment thereof or a microarray comprising microbeads, wherein the microbeads each bears multiple copies of a polynucleotide according to claims 28-31 or a fragment thereof and wherein the polynucleotide or fragment thereof is different from one bead to another.

The antibodies can be monoclonal or polyclonal antibodies. Monoclonal antibodies can be used for treating *Bordetella* infections. Also provided are immunological complexes comprising a protein or polypeptide of the invention and an antibody that specifically recognizes the protein or polypeptide.

Further, this invention provides a method for detecting infection by *Bordetella*. The method comprises providing a composition comprising a biological material suspected of being infected with *Bordetella* and assaying for the presence of a protein or polypeptide of the invention. The polypeptide can be assayed, for example, by electrophoresis or by immunoassay with antibodies that are immunologically reactive with the polypeptide.

The method can also comprise contacting the antigen with a biological fluid for a time and under conditions sufficient for the antigen and antibodies in the biological fluid to form an antigen-antibody complex, and detecting the formation of the complex. The method optionally can include measuring the formation of the antigen-antibody complex. In preferred embodiments, formation of antigen-antibody complex is detected by immunoassay based on Western blot technique, ELISA, indirect immunofluorescence assay, or immunoprecipitation assay.

Further, this invention provides a diagnostic kit for the detection of the presence or absence of antibodies, which bind a protein or polypeptide of the invention or mixtures thereof. The kit can comprise an antigen comprising the protein or polypeptide, or mixtures of the proteins and polypeptides, and means for detecting the formation of immune complexes between the antigen and antibodies. The means are present in an amount sufficient to perform the detection.

Another method of the invention for detecting the presence or absence of Bordetella comprises (1) contacting a sample suspected of containing genetic material of Bordetella with at least one nucleotide probe, and (2) detecting hybridization between the nucleotide probe and the genetic material in the sample. The nucleotide probe is complementary to a polynucleotide sequence of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

This invention will be described in greater detail with reference to the drawings in which:

Figure 1a is a map of the two regions of repeats, Region I and Region II, in the pertactin outer membrane protein of *Bordetella bronchiseptica*.

Figure 1b is an alignment of Region I of the pertactin outer membrane protein of different strains of *B. bronchiseptica*.

Figure 1c is an alignment of Region II of the pertactin outer membrane protein of different strains of *B. bronchiseptica*.

DETAILED DESCRIPTION OF THE INVENTION

It has been demonstrated previously that species-specific members of the pertactin family are outer-membrane proteins (OMPs). In *B. bronchiseptica*, pertactin is the product of the *pm* gene and is represented as a protein with an M_r of 68kDa (P.68), in *B. pertussis* as a protein with an M_r of 69kDa (P.69), and in *B. parapertussis* as a protein with an M_r of 70kDa (P.70). The nucleotide sequences of the pertactins of these three species are included in the accompanying Sequence Listing as SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively. The corresponding amino acid sequences encoded by these

nucleotide sequences are included in the sequence listing as SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively.

A comparison of the deduced protein sequences for the P.68, P.69. and P.70 proteins demonstrates the high degree of homology between the proteins. A comparison between the P.68 and P.70 proteins shows only 17 amino acid differences, while a similar comparison between P.68 and P.69 shows 80 differences, and 79 differences between P.69 and P.70. The majority of amino acid differences between the three deduced protein sequences occur in the number of repeat units in the two families of repeat sequences present in all three proteins. P.68 has three copies of the Gly-Gly-Xaa-Xaa-Pro repeat (i.e., GGXXP in Fig. 1b), while P.70 has four and P.69 five. Similarly, P.68 has seven Pro-Gln-Pro repeats (i.e., PQP in Fig. 1c), P.70 has nine and P.69 has five.

It has recently been shown that the PRN produced by clinical isolates of *B. pertussis* varies. Sequences of the *prn* gene of various clinical isolates revealed three major types of PRN variant. It has been suggested that epidemics in the Netherlands result from changes in the sequences of the genes encoding PRN and PT because the proteins present in the clinical isolates currently in circulation differ in sequence from those observed by the vaccinal strains used in this country.

An aim of the searches, which led to the present invention, was to analyze whether the PRN polymorphism observed in *B. pertussis* species also occurs in *B. parapertussis* and *B. bronchiseptica.* The two repeated regions of the *prn* genes of 10 *B. parapertussis* isolates of human origin and of 40 *B. bronchiseptica* isolates of animal or human origin were sequenced and compared. (Fig. 1a).

Table I contains a list of the isolates and corresponding pertactin types used in this invention.

TABLE I

Bordetella Species	Representative isolate	PRN regions I And II types /Number of isolates	Accession number,* region I, region II
BB	9.73H+	I-1, II-3/3	AJ250076, AJ250077
BB	LAPR	I-2, II-3/8	AJ250078, AJ250079
BB	5	1-2, II-4/8	AJ250080, AJ250081
BB	335	I-2, II-1/3	AJ250082, AJ250083
BB	CVGEO	I-2, II-5/6	AJ250084, AJ250085
BB	BBCH	I-2, II-6/4	AJ250086, AJ250087
BB	DEL	I-1, II-2/5	AJ250088, AJ25089

Bordetella Species	Representative isolate	PRN regions I And II types /Number of isolates	Accession number,* region I, region II
BB	CAT1	I-1, II-7/1	AJ250090, AJ250091
BB	286	I-3, II-8/1	AJ250093, AJ250092
BB	SEI	I-3, II-9/1	AJ250094, AJ250095
BPP	63.2	I-1, II-2/10	Identical to P24328

Species	Strain	PRN type	Accession number
BPP	CN2591	I-1, II-2	P24328
BB	CN7531	I-2, II-4	Q03035

Bordetella Species	Representative isolate	PRN regions I And II types /Number of isolates	Accession number,* region I, region II
Species	Strain or isolate	Allelic *prn* type	Accession number
BP	Tohama	*prnl*	AJ006158
BP	18323	*prn6*	AJ006152
BP	Hav	*prn2*	AJ007361
BP	Fr287	*prn3*	AJ006156

BB: *B. bronchiseptica*; BP: *B. pertussis*; BPP: *B. parapertussis*

*** EMBL Bank.**

In carrying out this invention, DNA was extracted, amplified by PCR, and sequenced, as previously described (3). Amplified PCR products were purified and sequenced by the ESGS company (ESGS, Cybergene group, Evry, France). Deduced amino acid sequences were analyzed with GCG software (Wisconsin Package Version 9.1, Genetics Computer Group, Madison, WI, USA). The deduced amino acid sequences of regions I and II were compared and multiple alignments of the amino acid sequences were created with the CLUSTAL W program of GCG (10), for each region (Fig. 1b,c).

No difference was found between the sequences of regions I and II of the PRN produced by the 10 *B. parapertussis* isolates and the published sequence (15). However, three different types were found among the 40 *B. bronchiseptica prn* genes analyzed with

differences in the number of repeats (1 to 3) in region I (Fig. 1b). The largest group corresponded to sequences with three copies of the repeated sequence, identical to the sequence previously reported (14). No correlation was found between the pattern of variation and the origin of the isolate.

A higher degree of variability was observed in the second repeated region of the *B. bronchiseptica* PRN (Fig. 1c). Nine variants were observed. Among these nine variants the number of repeats is from 6 to 9.

No *B. bronchiseptica* variants presented the same pattern as the *B. pertussis* variants. Furthermore, no unique association between one type of region I and one type of region II was observed. No observation was made in any of the three species of a pattern similar to those of the 18323 strain and the CZ isolate (3), which are considered to be intermediate between *B. pertussis, B. bronchiseptica*, and *parapertussis*. These data are consistent with *B. parapertussis* and *B. bronchiseptica prn* genes being more similar to each other than to the *B. pertussis prn* gene (1). No host specificity was observed with respect to PRN type.

It has been shown that region II plays an important role in the induction of protective immunity (6). The lack of cross-protection between PRN from *B. pertussis, B. parapertussis*, and *B. bronchiseptica* PRN is consistent with this, because the major differences between these proteins occur in this region. No variation in this region was observed for the PRN produced by *B. pertussis* isolates. These data suggest that thirty years of vaccination may have induced variation in one immunodominant repeat region, but not in the region most involved in the induction of protective immunity. Variation in *B. pertussis* PRN region II may indicate a decrease in *B. pertussis* vaccine efficacy.

In contrast, analysis of the PRN of *B. bronchiseptica* showed polymorphism in both regions. This may account for the inability of *B. bronchiseptica* vaccines to induce long-lasting protection. This polymorphism may also be linked to the ability of *B. bronchiseptica* to induce chronic infections (7, 8, 22). It may provide a means for this bacterium to escape host immune responses.

This invention, which resulted from these experiments and observations, thus involves compositions containing certain *Bordetella* pertactins and fragments thereof. These pertactins and pertactin fragments, as well as the polynucleotides that encode them, are useful in immunogenic compositions and in diagnostic applications.

In particular, this invention is the result of the discovery that there are different species of the full length pertactin of *Bordetella bronchiseptica,* namely, species containing 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and species of full length pertactin of *B. bronchiseptica* containing 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof, where XX can be FD, FG, or AV. These full length pertactins and mixtures of these pertactins in any combination of the repeating sequences are thus provided by this invention.

As used herein, the expression "pertactin of *Bordetella bronchiseptica*" means an outer membrane protein of *Bordetella bronchiseptica*, which is a virulence factor, and which has an apparent molecular weight of about 68 kDa, and which contains the two regions of *Bordetella bronchiseptica* pertactin known as Region I and Region II. Region I and Region II of the pertactins of different *Bordetella* strains are identified in brackets in SEQ ID NOS: 1 to 6. It will be understood that the pertactins of different isolates of *Bordetella bronchiseptica* may have amino acid sequences that differ from each other, for example, in Region I, Region II, or both Region I and Region II, as well as in other regions.

As used herein the expression "*Bordetella bronchiseptica* pertactin variants" means pertactins of Bordetella *bronchiseptica*, or fragments of pertactins of Bordetella *bronchiseptica* containing at least Region I, Region II, or both Region I and Region II, in which the pertactins of *Bordetella bronchiseptica* or the fragments thereof differ from each other in at least Region I, Region II, or both Region I and Region II, in their respective amino acid sequences. The following unique *Bordetella bronchiseptica* pertactin variants have been discovered and constitute part of this invention.

As used herein the expressions *Bordetella bronchiseptica* pertactin fragments", "*Bordetella parapertussis* pertactin fragments", and "*Bordetella pertussis* pertactin fragments" refer to polypeptides that are portions of full length pertactin proteins and are capable of inducing a humoral or immune response against *Bordetella* infections.

B. bronchiseptica pertactin – region I

```
I-1      QRATIRRGDAPAGGAVPGGAVPGGAVPG---------------GFGPLLDGWYGVDVSDSTVDLAQ  (SEQ  ID
NO: 7)
I-2      QRATIRRGDAPAGGAVPG-----GAVPG---------------GFGPLLDGWYGVDVSDSTVDLAQ  (SEQ  ID
NO: 8)
I-3      QRATIRRGDAPAGGGVPG-----GAVPG-----GFDPGGFGPGGFGPVLDGWYGVDVSGSTVELAQ  (SEQ  ID
NO: 9)
prn1     QRATIRRGDAPAGGAVPG-----GAVPG-----GAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQ  (SEQ  ID
NO: 10)
prn2     QRATIRRGDAPAGGAVPG-----GAVPGGFGPGGFGPGGFGPGGFGPVLDGWYGVDVSGSSVELAQ  (SEQ  ID
NO: 11)
prn3     QRATIRRGDAPAGGAVPG-----GAVPG-----GFGPGGFGPGGFGPVLDGWYGVDVSGSSVELAQ  (SEQ  ID
NO: 12)
prn4     QRATIRRGDAPAGGAVPG-----GAVPG----------GFGPGGFGPVLDGWYGVDVSGSSVELAQ  (SEQ  ID
NO: 13)
   **************.***      *****                 ****:**********.*:*:***
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B. bronchiseptica pertactin – region II

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II-1       GAKAPPAPKPAPQPGPQPGP----------QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID
NO: 14)
II-2       GAKAPPAPKPAPQPGPQPGPQPP--------QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID
NO: 15)
II-3       GAKAPPAPKPAPQPGPQPGPQPGPQPGPQPPQPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 16)
II-4       GAKAPPAPKPAPQPGPQPGPQPGP-------QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 17)
II-5       GAKAPPAPKPAPQPGPQPGPQPGPQP----PQPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 18)
II-6       GAKAPPAPKPAPQPGPQPGPQPPQPP--QPPQPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 19)
II-7       GAKAPPAPKPAPQPGPQP-P----------QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 20)
II-8       GAKVPPAPKPAPQPGPQP-PQPP--------QPPQPPQPQPQPQP--EAPAPQPPAGRELSAA (SEQ ID NO: 21)
II-9       GAKVPPAPKPAPQPGPQP-PQPP--------QPPQPPQPQPQPQPQPEAPAPQPPAGRELSAA (SEQ ID NO: 22)
prn1       GAKAPPAPKPAPQPGPQP---------------PQPPQP----QP--EAPAPQPPAGRELSAA (SEQ ID NO: 23)
prn6       GAKAPPAPKPAPQPGPQP-----------------PQP----QP--EAPAPQPPAGRELSAA (SEQ ID NO: 24)
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In specific embodiments, this invention includes a polypeptide comprising a sequence or a fragment of a sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22. The polypeptide can consist of the amino acids in SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22 or fragments thereof. The invention also includes polynucleotides encoding one of these polypeptides and a purified DNA or RNA sequence that hybridizes under moderate or high stringency conditions to the polynucleotides or at least to 15 nucleotides thereof.

As used herein, the expression "mixture of *Bordetella bronchiseptica* pertactin variants" means two or more *Bordetella bronchiseptica* pertactin variants in admixture in solid, liquid, emulsion, or suspension form. At least two of the *Bordetella bronchiseptica* pertactin variants in the mixture will, of course, differ from each other in at least Region I, Region II, or both Region I and Region II, in their respective amino acid sequences.

It will be immediately apparent that this invention provides polypeptide fragments of the pertactin of *B. bronchiseptica*, where the fragments comprise 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof or 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof. Mixtures of these polypeptide fragments in any combination of the repeating sequences are also within the scope of this invention.

When a polypeptide fragment of the invention comprises only Region I of a pertactin of *B. bronchiseptica*, the polypeptide fragment typically contains at least about 46 to about 56 amino acids, which includes the Region I repeat sequences. When the polypeptide fragment of the invention comprises only Region II, the polypeptide fragment typically contains at least about 48 to about 60 amino acids, which includes the Region II repeat sequences. When the polypeptide fragment of the invention comprises both Region I and Region II of *B. bronchiseptica*, the fragment typically contains at least about 906 to about 928 amino acids, which includes the repeat sequences of Regions I and II.

Thus, in one illustrative embodiment, this invention provides a composition comprising a mixture of *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises Region II of pertactin of *Bordetella*

bronchiseptica, and further wherein each *Bordetella bronchiseptica* pertactin variant comprises 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and the *Bordetella bronchiseptica* pertactin variants differ in the number of the repeating PQP amino acid sequences contained therein. The composition can also comprise pertactins of *Bordetella parapertussis*, *Bordetella pertussis*, or mixtures thereof. The polypeptide can be a full length pertactin or a fragment thereof.

In another embodiment, this invention provides a composition comprising a mixture of *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises Region I of a pertactin of *Bordetella bronchiseptica*, and further wherein each *Bordetella bronchiseptica* pertactin variant comprises 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof, and the at least two of the *Bordetella bronchiseptica* pertactin variants differ in the number of the repeating GGXXP amino acid sequences contained therein. This composition can also comprise pertactins of *Bordetella parapertussis*, *Bordetella pertussis*, or mixtures thereof. The *Bordetella bronchiseptica* pertactin variants can be full length or a fragment.

In a further embodiment, the invention provides a composition comprising a mixture of *Bordetella bronchiseptica* pertactin variants, wherein one of the *Bordetella bronchiseptica* pertactin variants comprises Region II of pertactin of *Bordetella bronchiseptica* having 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and another of the *Bordetella bronchiseptica* pertactin variants comprises Region I of pertactin of *Bordetella bronchiseptica* having 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof. This composition can also comprise pertactins of *Bordetella parapertussis*, *Bordetella pertussis*, or mixtures thereof. The *Bordetella bronchiseptica* pertactin variants can be full length or a fragment.

In a preferred embodiment, this invention provides a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9.

In another preferred embodiment, this invention provides a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, or SEQ ID NO: 22.

The compositions according to the invention cause a humoral immune response and a cellular immune response. After infection with *B. bronchiseptica*, there is induction of a humoral immunity and of a cellular immunity, as in the case of a *B. pertussis* and *B. parapertussis* infection. Furthermore, after vaccination with compositions of this invention, there is induction of a humoral and cellular type immunity similar to that induced after infection or reinfection.

In one embodiment of the invention there is provided a vaccinating composition comprising as active principle an immunogenic composition of the invention, in combination with a pharmaceutically acceptable vehicle and, where appropriate, with an adjuvant.

Like the whooping cough vaccines currently available on the market, the immunogenic composition according to the invention may be combined with other vaccinating active principles, for example, those of the vaccine against diphtheria, polio, or diseases caused by Haemophilus or, generally speaking, with any immunogenic constituent, for example, a particular inactivated pathogenic agent or toxin.

A vaccinating composition according to the invention can be species-specific and consequently capable of inducing protection against *B. pertussis* or *B. parapertussis* or *B. bronchiseptica*. Alternatively, it can be a mixture comprising as active principle an immunogenic composition against *B. bronchiseptica*, as defined above, and an immunogenic composition against *B. parapertussis* and/or *B. pertussis*.

As a result of recent techniques in molecular biology, a number of factors involved in the virulence of *B. pertussis* have been characterized and the regulation of their expression understood. These factors may be classified in two categories, those participating in the infectious syndrome (adhesins) and those playing a part in the toxin-induced syndrome (toxins). The adhesins and toxins relating to *Bordetella* can be included in the compositions of this invention. Examples of the adhesins are:

filamentous hemagglutinin or FHA, considered to play a major part in the adhesion of the bacterium to the ciliated epithelium;

the two agglutinogens or AGGs of *B. pertussis*, which enable strains to be classified in serotypes; and

pertussis toxin or PTX, a secreted type A-B toxin which, besides its cytopathogenic effects, participates in adhesion via its B subunit.

Examples of the toxins for use in the invention are:

pertussis toxin or PTX, which is secreted;

dermonecrotic toxin or DNT, which function has not yet been well characterized, and tracheal cytotoxin or TCT, a secreted small glycoprotein of the muramyl peptide family, derived from the peptidoglycan of the bacterium, which appear to act in concert to destroy the ciliated cells of the host's respiratory apparatus;

adenylate cyclase-hemolysin or Ac-Hly, a bifunctional protein possessing adenylate cyclase activity and hemolytic activity, which has been found to belong to the family of toxins termed "RTX" for "repeats in toxins".

Similarly, the factors involved in the virulence of *B. parapertussis* and *B. bronchiseptica* have been identified and can be included in the compositions of the invention.

The published results show that the acellular vaccines tested, monovalent (PTX), bivalent (PTX, FHA), trivalent (PTX, FHA, PRN), or pentavalent (PTX, FHA, PRN, AGG2, AGG3) induce very few side effects, are all immunogenic and all have an efficacy against the disease (according to WHO definition) which is greater than or equal to 70%. The compositions of the invention can be included in these vaccines and other acellular vaccines. For example, the immunogenic composition can further comprise at least one adhesin of *Bordetella* selected from the group consisting of FHA, AGG2, AGG3, and/or at least one toxin of *Bordetella* selected from the group consisting of PTX, DNT, TCT, and Ac-Hly.

The proteins, polypeptides, and compositions of this invention can be in purified form. The term "purified" as used herein, means that the pertactins and fragments thereof are essentially free of association with other proteins or polypeptides, for example, as a purification product of recombinant host cell culture or as a purified product from a non-recombinant source. The term "substantially purified" as used herein, refers to a mixture that contains pertactins or fragments thereof and is essentially free of association with other proteins or polypeptides, but for the presence of known proteins that can be removed using a specific antibody, and which substantially purified pertactin polypeptides can be used as

antigens.

Within an aspect of the invention, the pertactin and fragments thereof can be utilized to prepare antibodies that specifically bind to pertactin polypeptides. The term "antibodies" is meant to include polyclonal antibodies, monoclonal antibodies, fragments thereof, such as F(ab')2 and Fab fragments, as well as any recombinantly produced binding partners. Antibodies are defined to be specifically binding if they bind pertactins and fragments thereof with a K_a of greater than or equal to about 10^7 M^{-1}. Affinities of binding partners or antibodies can be readily determined using conventional techniques, for example, those described by Scatchard et al., *Ann. N.Y Acad. Sci., 51*:660 (1949). Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice, or rats, using procedures that are well known in the art.

The invention further encompasses isolated fragments and oligonucleotides derived from the nucleotide sequence of the pertactins *B. bronchiseptica, B. pertussis* and *B. parapertussis* (SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3) encoding 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and/or 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof. The invention also encompasses polypeptides encoded by these fragments and oligonucleotides. Mixtures can comprise nucleotide sequences containing repeating sequences in which each entity in the mixture is independently selected from the polynucleotides of the invention.

Nucleic acid sequences within the scope of the invention include isolated DNA and RNA sequences that hybridize to the native pertactin nucleic acids disclosed herein under conditions of moderate or severe stringency, and which encode pertactin polypeptides. As used herein, conditions of moderate stringency, as known to those having ordinary skill in the art, and as defined by Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2 ed. Vol. 1, pp. 1.101-104, Cold Spring Harbor Laboratory Press, (1989), include use of a prewashing solution for the nitrocellulose filters 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization conditions of 50% formamide, 6X SSC at 42 C (or other similar hybridization solution, such as Stark's solution, in 50% formamide at 42 C), and washing conditions of about 60 C, 0.5X SSC, 0.1% SDS. Conditions of high stringency are defined as hybridization conditions as above, and with washing at 68 C, 0.2X SSC, 0.1% SDS. The skilled artisan will recognize that the temperature and wash solution salt

concentration can be adjusted as necessary according to factors such as the length of the probe.

Due to the known degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, a DNA sequence can vary and still encode a pertactin polypeptide having the amino acid sequence of SEQ ID NO:7 through SEQ ID NO:24. Such variant DNA sequences can result from silent mutations (e.g., occurring during PCR amplification), or can be the product of deliberate mutagenesis of a native sequence.

The invention thus provides equivalent isolated DNA sequences, encoding pertactin polypeptides, selected from: (a) DNA derived from the coding region of a native pertactin gene; (b) cDNA comprising the nucleotide sequence of SEQ ID NO:7 through SEQ ID NO:24; (c) DNA capable of hybridization to a DNA of (a) under conditions of moderate stringency and which encode pertactin polypeptides; and (d) DNA which is degenerate as a result of the genetic code to a DNA defined in (a), (b) or (c) and which encodes pertactin polypeptides. Pertactin polypeptides encoded by such DNA equivalent sequences are encompassed by the invention.

It will be understood that the present invention is intended to encompass the previously described proteins and polypeptides in isolated or purified form, whether obtained using the techniques described herein or other methods. In a preferred embodiment of this invention, the pertactin polypeptides are substantially free of human or other animal tissue and human or other animal tissue components, nucleic acids, extraneous proteins and lipids, and adventitious microorganisms, such as bacteria and viruses. It will also be understood that the invention encompasses equivalent proteins having substantially the same biological and immunogenic properties. Thus, this invention is intended to cover serotypic variants of the polypeptides of the invention.

Depending on the use to be made of the pertactin polypeptides of the invention, it may be desirable to label them. Examples of suitable labels are radioactive labels, enzymatic labels, fluorescent labels, chemiluminescent labels, and chromophores. The methods for labeling do not differ in essence from those widely used for labeling immunoglobulin. The need to label may be avoided by using labeled antibody to the antigen of the invention or anti-immunoglobulin to the antibodies to the antigen as an indirect marker.

Once the pertactin polypeptides of the invention have been obtained, they can be used to produce polyclonal and monoclonal antibodies reactive therewith. Thus, a protein or polypeptide of the invention can be used to immunize an animal host by techniques known in the art. Such techniques usually involve inoculation, but they may involve other modes of administration. A sufficient amount of the protein or the polypeptide is administered to create an immunogenic response in the animal host. Any host that produces antibodies to the antigen of the invention can be used. Once the animal has been immunized and sufficient time has passed for it to begin producing antibodies to the antigen, polyclonal antibodies can be recovered. The general method comprises removing blood from the animal and separating the serum from the blood. The serum, which contains antibodies to the antigen, can be used as an antiserum to the antigen. Alternatively, the antibodies can be recovered from the serum. Affinity purification is a preferred technique for recovering purified polyclonal antibodies to the antigen, from the serum.

Monoclonal antibodies to the antigens of the invention can also be prepared. One method for producing monoclonal antibodies reactive with the antigens comprises the steps of immunizing a host with the antigen; recovering antibody producing cells from the spleen of the host; fusing the antibody producing cells with myeloma cells deficient in the enzyme hypoxanthine-guanine phosphoribosyl transferase to form hybridomas; select at least one of the hybridomas by growth in a medium comprising hypoxanthine, aminopterin, and thymidine; identifying at least one of the hybridomas that produces an antibody to the antigen, culturing the identified hybridoma to produce antibody in a recoverable quantity; and recovering the antibodies produced by the cultured hybridoma.

These polyclonal or monoclonal antibodies can be used in a variety of applications. Among these is the neutralization of corresponding proteins. They can also be used to detect *Bordetella* antigens in biological preparations or in purifying corresponding proteins, glycoproteins, or mixtures thereof, for example when used in a affinity chromatographic columns.

The pertactin polypeptides of the invention can be used as antigens to identify antibodies to *Bordetella* in materials and to determine the concentration of the antibodies in those materials. Thus, the antigens can be used for qualitative or quantitative determination of *Bordetella* in a material. Such materials, of course, include human or

other animal tissue and human or other animal cells, as well as biological fluids, such as human or other animal body fluids, including human sera. When used as a reagent in an immunoassay for determining the presence or concentration of the antibodies to *Bordetella*, the antigens of the present invention provide an assay that is convenient, rapid, sensitive, and specific.

More particularly, the antigens of the invention can be employed for the detection of *Bordetella* by means of immunoassays that are well known for use in detecting or quantifying humoral components in fluids. Thus, antigen-antibody interactions can be directly observed or determined by secondary reactions, such as precipitation or agglutination. In addition, immunoelectrophoresis techniques can also be employed. For example, the classic combination of electrophoresis in agar followed by reaction with anti-serum can be utilized, as well as two-dimensional electrophoresis, rocket electrophoresis, and immunolabeling of polyacrylamide gel patterns (Western Blot or immunoblot.) Other immunoassays in which the antigens of the present invention can be employed include, but are not limited to, radioimmunoassay, competitive immunoprecipitation assay, enzyme immunoassay, and immunofluorescence assay. It will be understood that turbidimetric, colorimetric, and nephelometric techniques can be employed. An immunoassay based on Western Blot technique is preferred.

Immunoassays can be carried out by immobilizing one of the immunoreagents, either an antigen of the invention or an antibody of the invention to the antigen, on a carrier surface while retaining immunoreactivity of the reagent. The reciprocal immunoreagent can be unlabeled or labeled in such a manner that immunoreactivity is also retained. These techniques are especially suitable for use in enzyme immunoassays, such as enzyme linked immunosorbent assay (ELISA) and competitive inhibition enzyme immunoassay (CIEIA).

When either the antigen of the invention or antibody to the antigen is attached to a solid support, the support is usually a glass or plastic material. Plastic materials molded in the form of plates, tubes, beads, or disks are preferred. Examples of suitable plastic materials are polystyrene and polyvinyl chloride. If the immunoreagent does not readily bind to the solid support, a carrier material can be interposed between the reagent and the support. Examples of suitable carrier materials are proteins, such as bovine serum albumin, or

chemical reagents, such as gluteraldehyde or urea. Coating of the solid phase can be carried out using conventional techniques.

The invention provides immunogenic pertactin polypeptides, and more particularly, protective polypeptides for use in the preparation of vaccine compositions against *Bordetella*. These polypeptides can thus be employed as vaccines by administering the polypeptides to a mammal susceptible to *Bordetella* infection. Conventional modes of administration can be employed. For example, administration can be carried out by oral, respiratory, or parenteral routes. Intradermal, subcutaneous, and intramuscular routes of administration are preferred when the vaccine is administered parenterally.

The major purpose of the immune response in a *Bordetella*-infected mammal is to inactivate the *Bordetella* and to eliminate *Bordetella* infected cells that have the potential to release infectious virus. The B-cell arm of the immune response has the major responsibility for inactivating *Bordetella*. The principal manner in which this is achieved is by neutralization of infectivity. Another major mechanism for destruction of the *Bordetella*-infected cells is provided by cytotoxic T lymphocytes (CTL) that recognize pertactin antigens expressed in combination with class I histocompatibility antigens at the cell surface. The CTLs recognize pertactin polypeptides processed within cells from a pertactin protein that is produced, for example, by the infected cell or that is internalized by a phagocytic cell. Thus, this invention can be employed to stimulate a B-cell response to pertactin polypeptides, as well as immunity mediated by a CTL response following infection. The CTL response can play an important role in mediating recovery from primary *Bordetella* infection and in accelerating recovery during subsequent infections.

The ability of the pertactin polypeptides and vaccines of the invention to induce protective levels of neutralizing antibody in a host can be enhanced by emulsification with an adjuvant, incorporating in a liposome, coupling to a suitable carrier, or by combinations of these techniques. For example, the pertactin polypeptides of the invention can be administered with a conventional adjuvant, such as aluminum phosphate and aluminum hydroxide gel, in an amount sufficient to potentiate humoral or cell-mediated immune response in the host. Similarly, the pertactin polypeptides can be bound to lipid membranes or incorporated in lipid membranes to form liposomes. The use of nonpyrogenic lipids free of nucleic acids and other extraneous matter can be employed for

this purpose.

The immunization schedule will depend upon several factors, such as the susceptibility of the host to infection and the age of the host. A single dose of the vaccine of the invention can be administered to the host or a primary course of immunization can be followed in which several doses at intervals of time are administered. Subsequent doses used as boosters can be administered as need following the primary course.

The pertactin proteins, polypeptides, and vaccines of the invention can be administered to the host in an amount sufficient to prevent or inhibit *Bordetella* infection or replication *in vivo*. In any event, the amount administered should be at least sufficient to protect the host against substantial immunosuppression, even though *Bordetella* infection may not be entirely prevented. An immunogenic response can be obtained by administering the proteins or polypeptides of the invention to the host in an amount of, for example, about 1 to about 50 micrograms antigen per kilogram of body weight, preferably about 5 to about 10 micrograms antigen per kilogram of body weight. The proteins, polypeptides, and vaccines of the invention can be administered together with a physiologically acceptable carrier. For example, a diluent, such as water or a saline solution, can be employed.

Another aspect of the invention includes administering any combination of the nucleic acids encoding pertactin polypeptides, the proteins, and polypeptides *per se*, with or without carrier molecules, to an individual. The individual can be an animal. As used herein, the term "animal" means a mammal, and preferably, the mammal is selected from the group consisting of a human, a rabbit, a mouse, a dog, a cat, a bovine, a pig, and a horse. In an especially preferred embodiment, the mammal is a human.

The methods of treating include administering immunogenic compositions comprising pertactin proteins or polypeptides, and compositions comprising nucleic acids encoding pertactin proteins or polypeptides as well. Those of skill in the art are cognizant of the concept, application, and effectiveness of nucleic acid vaccines (*e.g.*, DNA vaccines) and nucleic acid vaccine technology as well as protein and polypeptide based technologies. The nucleic acid based technology allows the administration of nucleic acids encoding pertactin polypeptides, naked or encapsulated, directly to tissues and cells without the need for production of encoded proteins prior to administration. The technology is based on the ability of these nucleic acids to be taken up by cells of the recipient organism and

expressed to produce an immunogenic determinant to which the recipient's immune system responds. Typically, the expressed antigens are displayed on the surface of cells that have taken up and expressed the nucleic acids, but expression and export of the encoded antigens into the circulatory system of the recipient individual is also within the scope of the present invention. Such nucleic acid vaccine technology includes, but is not limited to, delivery of naked DNA and RNA and delivery of expression vectors encoding pertactin polypeptides. Although the technology is termed "vaccine", it is equally applicable to immunogenic compositions that do not result in a protective response. Such non-protection inducing compositions and methods are encompassed within the present invention.

Although it is within the present invention to deliver nucleic acids encoding pertactin polypeptides and carrier molecules as naked nucleic acid, the present invention also encompasses delivery of nucleic acids as part of larger or more complex compositions. Included among these delivery systems are viruses, virus-like particles, or bacteria containing the nucleic acid encoding pertactin polypeptides. Also, complexes of the invention's nucleic acids and carrier molecules with cell permeabilizing compounds, such as liposomes, are included within the scope of the invention. Other compounds, such as molecular vectors (EP 696,191, Samain et al.) and delivery systems for nucleic acid vaccines are known to the skilled artisan and exemplified in, for example, WO 93 06223 and WO 90 11092, U.S. 5,580,859, and U.S. 5,589,466 (Vical patents), which are incorporated by reference herein, and can be made and used without undue or excessive experimentation.

To further achieve the objects and in accordance with the purposes of the present invention, a kit capable of diagnosing a *Bordetella* infection is described. This kit, in one embodiment, contains the DNA sequences of this invention, which are capable of hybridizing to bacterial RNA or analogous DNA sequences to indicate the presence of a *Bordetella* infection. Different diagnostic techniques can be used which include, but are not limited to: (1) Southern blot procedures to identify cellular DNA which may or may not be digested with restriction enzymes; (2) Northern blot techniques to identify RNA extracted from cells; and (3) dot blot techniques, i.e., direct filtration of the sample through a membrane, such as nitrocellulose or nylon, without previous separation on agarose gel.

Suitable material for dot blot technique could be obtained from body fluids including, but not limited to, serum and plasma, supernatants from culture cells, or cytoplasmic extracts obtained after cell lysis and removal of membranes and nuclei of the cells by centrifugation.

Following are references of the strains used in the search concerning the present invention:

- 9.73H+5, DEL, SEI: Infect Immun. (1993) 61"4072-4078. Gueirard, P. and Guiso, N., filed with CNCM on May 12, 1989, No. 858.

- CVGEO identical to strain CVHAI 286, 335: Microbiol. (1997) 143:1433-1441. Le Blay, K. et al.

- 63.2: CIP - Lab. Ident., Inst. Pasteur, Paris, France - J. Clin. Microbiol., 1993, 31, 2745

- TI: CIP81.32 - Lab. Ident., Inst. Pasteur, Paris, France - J. Clin. Microbiol., 1993, 31, 2746

- Fr287: Vaccine (1999) 17:2651:2660. Boursaux-Eude, C. et al.

- 18232: ref OMS: ATCC97.97 (CIP63.1).

B. bronchiseptica p.68 pertactin gene [SEQ ID NO:1]

atcgatgatg cgtcgctgta acacggcaaa taccgtgcat tgcagcggtt ctggatggcg
ttcttcgtac gtttgctgcg cccattcttc cctgttccat cgcggtgcgg ccatggcggg
cgtctgctct tcacccggca tccaatgaac atgtctctgt cacgcattgt cttggcggcg
cccctgcgcc gcaccacact ggccatggcg ctgggcgcgc tgggcgccgc gcccgccgcg
tacgccgact ggaacaacca gtccatcatc aaggccggcg agcgccagca cggcatccac
atcaagcaaa gcgatggcgc cggcgtacgg accgccaccg gaacgaccat caaggtaagc
ggtcgtcagg cccagggcgt cctgctggaa aatcccgcgg ccgagctgcg gttccagaac
ggcagcgtca cgtcttcggg acagctgttc gacgaaggcg tccggcgctt tctgggcacc
gtcaccgtca aggccggcaa gctggtcgcc gatcacgcca cgctggccaa cgtcagcgac
acccgggacg acgacggcat cgcgctctat gtggccggcg agcaggccca ggccagcatc
gccgacagca ccctgcaggg cgcgggcggc gtgcgggtcg agcgcggcgc caatgtcacg
gtccaacgca gcaccatcgt tgacgggggc ttgcatatcg gcaccctgca gccgctgcag
ccggaagacc ttccgcccag ccgggtggtg ctgggcgaca ccagcgtgac cgccgtgccc
gccagcggcg cgcccgcggc ggtgtctgta ttcggggcca atgagcttac ggttgatggc
gggcacatca ccgggggcg ggcagcgggg gtggcggcca tggacggggc gatcgtgcat
ctg[cagcgcg cgacgatacg gcgggggac gcgcctgccg gcggtgcggt tccaggcggt
gctgttcccg gcggcttcgg ccccctcctt gacggctggt atggcgtgga tgtatcggat
tccaccgtgg acctcgctca g]*tcgatcgtc gaggcgccgc agctgggcgc
cgcgatccgg gcgggccgcg gcgccagggt gacggtgtcg ggcggcagct tgtccgcacc
gcacggcaat gtcatcgaga ccggcggcgg cgcgcgtcgc ttcccgcctc cggcctcgcc
cctgtcgatc accttgcagg cgggcgcacg ggcgcagggg agggcgctgc tgtaccgggt
cctgccggag cccgtgaagc tgacgctggc gggcggcgcc caggggcagg gcgacatcgt
cgcgacggag ctgcctccca ttccaggcgc gtcgagcggg ccgctcgacg tggcgctggc
cagccaggcc

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* Region I

cgatggacgg gcgctacccg cgcggtcgac tcgctgtcca tcgacaacgc cacctgggtc
atgacggaca actcgaacgt cggcgcgctg cggctggcca gcgacggcag cgtcgatttc
cagcagccgg ccgaagctgg gcggttcaag tgcctgatgg tcgatacgct ggcgggttcg
gggctgttcc gcatgaatgt cttcgcggac ctggggctga gcgacaagct ggtcgtcatg
cgggacgcca gcggccagca caggctgttg gtccgcaaca gcggcagcga gccggccagc
ggcaacacca tgctgctggt gcagacgcca cgaggcagcg cggcgacctt tacccttgcc
aacaaggacg gcaaggtcga tatcggtacc taccgctatc gattggccgc caacggcaat
gggcagtgga gcctggtg[gg cgcgaaggcg ccgccggcgc ccaagcccgc gccgcagccc
ggtccccagc ccggtcccca gccgccgcag ccgccgcagc cgccgcagcc gccacagagg
cagccggaag cgccggcgcc gcaaccgccg gcgggcaggg agttgtccgc
cgcc]**gccaac gcggcggtca acacgggtgg ggtgggcctg gccagcacgc
tctggtacgc cgaaagcaat gcgttgtcca agcgcctggg cgagttgcgc ctgaatccgg
acgccggcgg cgcttggggc cgcggcttcg cgcaacgcca gcaactggac aaccgcgccg
ggcggcgctt cgaccagaag gtggccggct tcgagctggg cgccgaccac gcggtggcgg
tggccggcgg gcgctggcac ctgggcgggc tggccggcta tacgcgcggc gaccgcggct
ttaccggcga cggcggcggc cacaccgaca gcgtgcatgt cgggggctat gccacctata
tcgccaacag cggtttctac ctggacgcga cgctgcgcgc cagccgcctc gaaaatgact
tcaaggtggc gggcagcgat gggtacgcgg tcaagggcaa gtaccgcacc catggggtag
gcgcctcgct cgaggcgggc cggcgcttcg cccatgccga cggctggttc ctcgagccgc
aggccgagct ggcggtgttc cgggtcggcg gcggttcgta ccgcgcggcc aatggcctgc
gggtgcgcga cgaaggcggc agctcggtgc tgggtcgcct gggcctggag gtcggcaagc
gcatcgaact ggcaggcggc aggcaggtgc agccatacat caaggccagc gtgctgcagg
agttcgacgg cgcgggtacg gtacgcacca acggcatcgc gcaccgcacc gaactgcgcg
gcacgcgcgc cgaactgggc ctgggcatgg ccgccgcgct gggccgcggc cacagcctgt
atgcctcgta cgagtactcc aagggcccga agctggccat gccgtggacc ttccacgcgg
gctaccggta cagctggtaa

** Region II

agcgagaagg gtccatcccc ccgcggggga gattttcctg gaggttggcc ggtgccagtc
tccaggctca ggcggccagg gcgtgcgggc cgggcaggcc gtgctggtgc tggccgaacc

B. bronchiseptica p.68 pertactin protein [SEQ ID NO:4]

MNMSLSRIVL AAPLRRTTLA MALGALGAAP AAYADWNNQS IIKAGERQHG IHIKQSDGAG
VRTATGTTIK VSGRQAQGVL LENPAAELRF QNGSVTSSGQ LFDEGVRRFL GTVTVKAGKL
VADHATLANV SDTRDDDGIA LYVAGEQAQA SIADSTLQGA GGVRVERGAN VTVQRSTIVD
GGLHIGTLQP LQPEDLPPSR VVLGDTSVTA VPASGAPAAV SVFGANELTV DGGHITGGRA
AGVAAMDGAI VHL[QRATIRR GDAPAGGAVP GGAVPGGFGP LLDGWYGVDV
SDSTVDLAQ]*S IVEAPQLGAA IRAGRGARVT VSGGSLSAPH GNVIETGGGA
RRFPPPASPL SITLQAGARA QGRALLYRVL PEPVKLTLAG GAQGQGDIVA TELPPIPGAS
SGPLDVALAS QARWTGATRA VDSLSIDNAT WVMTDNSNVG ALRLASDGSV DFQQPAEAGR
FKCLMVDTLA GSGLFRMNVF ADLGLSDKLV VMRDASGQHR LLVRNSGSEP ASGNTMLLVQ
TPRGSAATFT LANKDGKVDI GTYRYRLAAN GNGQWSLV[GA KAPPAPKPAP QPGPQPGPQP
PQPPQPPQPP QRQPEAPAPQ PPAGRELSAA]** ANAAVNTGGV GLASTLWYAE
SNALSKRLGE LRLNPDAGGA WGRGFAQRQQ LDNRAGRRFD QKVAGFELGA DHAVAVAGGR
WHLGGLAGYT RGDRGFTGDG GGHTDSVHVG GYATYIANSG FYLDATLRAS RLENDFKVAG
SDGYAVKGKY RTHGVGASLE AGRRFAHADG WFLEPQAELA VFRVGGGSYR AANGLRVRDE
GGSSVLGRLG LEVGKRIELA GGRQVQPYIK ASVLQEFDGA GTVRTNGIAH RTELRGTRAE
LGLGMAAALG RGHSLYASYE YSKGPKLAMP WTFHAGYRYS W

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* Region I

** Region II

B. pertussis p.69 gene [SEQ ID NO:2]

atgaacatgt ctctgtcacg cattgtcaag gcggcgcccc tgcgccgcac cacgctggcc
atggcgctgg gcgcgctggg cgccgccccg gcggcgcatg ccgactggaa caaccagtcc
atcgtcaaga ccggtgagcg ccagcatggc atccatatcc agggctccga cccgggcggc
gtacggaccg ccagcggaac caccatcaag gtaagcggcc gtcaggccca gggcatcctg
ctagaaaatc ccgcggccga gctgcagttc cggaacggca gtgtcacgtc gtcgggacag
ttgtccgacg atggcatccg gcgctttctg ggcaccgtca ccgtcaaggc cggcaagctg
gtcgccgatc acgccacgct ggccaacgtt ggcgacacct gggacgacga cggcatcgcg
ctctatgtgg ccggcgaaca ggcccaggcc agcatcgccg acagcaccct gcagggcgct
ggcggcgtgc agatcgagcg cggcgccaat gtcacggtcc aacgcagcgc catcgtcgac
gggggcttgc atatcggcgc cctgcagtca ttgcagccgg aagaccttcc gcccagccgg
gtggtgctgc gcgacaccaa cgtgaccgcc gtgcccgcca gcggcgcgcc cgcggcggtg
tctgtgttgg gggccagtga gcttacgctc gacggcgggc acatcaccgg cgggcgggca
gcgggggtgg cggccatgca aggggcggtc gtgcatctg[c agcgcgcgac gatacggcgc
ggggacgcgc ctgccggcgg tgcggttccc ggcggtgcgg ttcccggtgg tgcggttccc
ggcggcttcg gtcccggcgg cttcggtccc gtcctcgacg gctggtatgg cgtggacgta
tcgggctcca gcgtggagct cgcccag]*tcg atcgtcgagg cgccggagct
gggcgccgca atccgggtgg gccgcggcgc cagggtgacg gtgtcgggcg gcagcttgtc
cgcaccgcac ggcaatgtca tcgagaccgg cggcgcgcgt cgctttgcgc ctcaagccgc
gcccctgtcg atcaccttgc aggccggcgc gcatgcccag gggaaagcgc tgctgtaccg
ggtcctgccg gagcccgtga agctgacgct gaccgggggc gccgatgcgc agggcgacat
cgtcgcgacg gagctgccct ccattcccgg cacgtcgatc gggccgctcg acgtggcgct
ggccagccag gcccgatgga cgggcgctac ccgcgcggtc gactcgctgt ccatcgacaa
cgccacctgg gtcatgacgg acaactcgaa cgtcggtgcg ctacggctgg ccagcgacgg
cagcgtcgat

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* Region I

ttccagcagc cggccgaagc tgggcggttc aaggtcctga cggtcaatac gctggcgggt
tcggggctgt tccgcatgaa tgtcttcgcg gacctggggc tgagcgacaa gctggtcgtc
atgcaggacg ccagcggcca gcacaggctg tgggtccgca acagcggcag cgagccggcc
agcgccaaca ccctgctgct ggtgcagacg ccacgaggca gcgcggcgac ctttaccctt
gccaacaagg acggcaaggt cgatatcggt acctatcgct atcgattggc cgccaacggc
aatgggcagt ggagcctggt g[ggcgcgaag gcgccgccgg cgcccaagcc cgcgccgcag
ccgggtcccc agccgccgca gccgccgcag ccgcagccgg aagcgccggc gccgcaaccg
ccggcgggca gggagttgtc cgccgcc]**gcc aacgcggcgg tcaacacggg
tggggtgggc ctggccagca cgctctggta cgccgaaagc aatgcgttgt ccaagcgcct
gggcgagttg cgcctgaatc cggacgccgg cggcgcctgg ggccgcggct tcgcgcaacg
ccagcagctg gacaaccgcg ccgggcggcg cttcgaccag aaggtggccg gcttcgagct
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tgtcgggggc tatgccacat atatcgccga cagcggtttc tacctggacg cgacgctgcg
cgccagccgc ctggagaatg acttcaaggt ggcgggcagc gacgggtacg cggtcaaggg
caagtaccgc acccatgggg tgggcgcctc gctcgaggcg ggccggcgct ttacccatgc
cgacggctgg ttcctcgagc cgcaggccga gctggcggta ttccgggccg gcggcggtgc
gtaccgcgcg gccaacggcc tgcgggtgcg cgacgaaggc ggcagctcgg tgctgggtcg
cctgggcctg gaggtcggca agcgcatcga actggcaggc ggcaggcagg tgcagccata
catcaaggcc agcgtgctgc aggagttcga cggcgcgggt acggtacaca ccaacggcat
cgcgcaccgc accgaactgc gcggcacgcg cgccgaactg ggcctgggca tggccgccgc
gctgggccgc ggccacagcc tgtatgcctc gtacgagtac tccaagggcc cgaagctggc
catgccgtgg accttccacg cgggctaccg gtacagctgg taa

** Region II

B. pertussis p.69 protein [SEQ ID NO:5]

MNMSLSRIVK AAPLRRTTLA MALGALGAAP AAHADWNNQS IVKTGERQHG IHIQGSDPGG
VRTASGTTIK VSGRQAQGIL LENPAAELQF RNGSVTSSGQ LSDDGIRRFL GTVTVKAGKL
VADHATLANV GDTWDDDGIA LYVAGEQAQA SIADSTLQGA GGVQIERGAN VTVQRSAIVD
GGLHIGALQS LQPEDLPPSR VVLRDTNVTA VPASGAPAAV SVLGASELTL DGGHITGGRA
AGVAAMQGAV VHL[QRATIRR GDAPAGGAVP GGAVPGGAVP GGFGPGGFGP VLDGWYGVDV
SGSSVELAQ]*S IVEAPELGAA IRVGRGARVT VSGGSLSAPH GNVIETGGAR
RFAPQAAPLS ITLQAGAHAQ GKALLYRVLP EPVKLTLTGG ADAQGDIVAT ELPSIPGTSI
GPLDVALASQ ARWTGATRAV DSLSIDNATW VMTDNSNVGA LRLASDGSVD FQQPAEAGRF
KVLTVNTLAG SGLFRMNVFA DLGLSDKLVV MQDASGQHRL WVRNSGSEPA SANTLLLVQT
PRGSAATFTL ANKDGKVDIG TYRYRLAANG NGQWSLV[GAK APPAPKPAPQ PGPQPPQPPQ
PQPEAPAPQP PAGRELSAA]**A NAAVNTGGVG LASTLWYAES NALSKRLGEL
RLNPDAGGAW GRGFAQRQQL DNRAGRRFDQ KVAGFELGAD HAVAVAGGRW HLGGLAGYTR
GDRGFTGDGG GHTDSVHVGG YATYIADSGF YLDATLRASR LENDFKVAGS DGYAVKGKYR
THGVGASLEA GRRFTHADGW FLEPQAELAV FRAGGGAYRA ANGLRVRDEG GSSVLGRLGL
EVGKRIELAG GRQVQPYIKA SVLQEFDGAG TVHTNGIAHR TELRGTRAEL GLGMAAALGR
GHSLYASYEY SKGPKLAMPW TFHAGYRYSW

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* Region I

** Region II

B. parapertussis p.70 gene [SEQ ID NO:3]

atcgatgatg cgtcgctgta acacggcaaa taccgtgcat tgcagcggtt ctggatggcg
ttcttcgtac gtttgctgcg cccattcttc cctgttccat cgcggtgcgg gcatggcggg
cgtctgctct tcacccggca tccaatgaac atgtctctgt cacgcattgt caaggcggcg
cccctgcgcc gcaccacact ggccatggcg ctgggcgcgc tgggcgccgc gcccgccgcg
tacgccgact ggaacaacca gtccatcatc aaggccggcg agcgccagca cggcatccac
atcaagcaaa gcgatggcgc cggcgtacgg accgccaccg gaacgaccat caaggtaagc
ggtcgtcagg cccagggcgt cctgctggaa aatcccgcgg ccgagctgcg gttccagaac
ggcagcgtca cgtcttcggg acagctgttc gacgaaggcg tccggcgctt tctgggcacc
gtcaccgtca aggccggcaa gctggtcgcc gatcacgcca cgctggccaa cgtcagcgac
acccgggacg acgacggcat cgcgctctat gtggccggcg agcaggccca ggccagcatc
gccgacagca ccctgcaggg cgcgggcggc gtgcgggtcg agcgcggcgc caatgtcacg
gtccaacgca gcaccatcgt tgacgggggc ttgcatatcg gcaccctgca gccgctgcag

ccggaagacc ttccgcccag ccgggtggtg ctgggcgaca ccagcgtgac cgccgtgccc
gccagcggcg cgcccgcggc ggtgtttgta ttcggggcca atgagcttac ggttgatggc
gggcacatca ccgggggggcg ggcagcgggg gtggcggcca tggacggggc gatcgtgcat
ctg[cagcgcg cgacgatacg gcgggggggac gcgcctgccg gcggtgcggt tccaggcggt
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gtggatgtat cggactccac cgtggacctc gctcag]*tcga tcgtcgaggc
gccgcagctg ggcgccgcga tccgggcggg ccgcggcgcc agggtgacgg tgtcgggcgg
cagcttgtcc gcaccgcacg gcaatgtcat cgagaccggc ggcggtgcgc gtcgcttccc
gcctccggcc tcgcccctgt cgatcacctt gcaggcgggc gcacgggcgc aggggagggc
gctgctgtac cgggtcctgc cggagcccgt gaagctgacg ctggcgggcg gcgcccaggg
gcagggcgac atcgtcgcga cggagctgcc tcccattcca ggcgcgtcga gcgggccgct
cgacgtggcg ctggccagcc aggcccgatg gacgggcgct acccgcgcgg tcgactcgct
gtccatcgac aacgccacct gggtcatgac ggacaactcg aacgtcggcg cgctgcggct
ggccagcgac ggcagcgtcg atttccagca gccggccgaa gctgggcggt tcaaggtcct
gatggtcgat acgctggcgg gttcggggct gttccgcatg aatgtcttcg cggacctggg
gctgagcgac aagctggtcg tcatgcggga cgccagcggc cagcacaggc tgtgggtccg
caacagcggc agcgagccgg ccagcggcaa caccatgctg ctggtgcaga cgccacgagg
cagcgcggcg

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* Region I

acctttaccc ttgccaacaa ggacggcaag gtcgatatcg gtacctaccg ctatcgattg
gccgccaacg gcaatgggca gtggagcctg gtg[ggcgcga aggcgccgcc ggcgcccaag
cccgcgccgc agcccggtcc ccagcccggt ccccagccgc cgcagccgcc gcagccgccg
cagccgccgc agccgccgca gccgccacag aggcagccgg aagcgccggc gccgcaaccg
ccggcgggca gggagttgtc cgccgcc]**gcc aacgcggcgg tcaacacggg
tggggtgggc ctggccagca cgctctggta cgccgaaagc aatgcgttgt ccaagcgcct
gggcgagttg cgcctgaatc cggacgccgg cggcgcttgg ggccgcggct tcgcgcaacg
ccagcaactg gacaaccgcg ccgggcggcg cttcgaccag aaggtggccg gcttcgagct
gggcgccgac cacgcggtgg cggtggccgg cgggcgctgg cacctgggcg ggctggccgg
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tgtcgggggc tatgccacct atatcgccaa cagcggtttc tacctggacg cgacgctgcg
cgccagccgc ctcgaaaatg acttcaaggt ggcgggcagc gatgggtacg cggtcaaggg
caagtaccgc acccatgggg taggcgtctc gctcgaggcg ggccggcgct tcgcccatgc
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gtaccgcgcg gccaatggcc tgcgggtgcg cgacgaaggc ggcagctcgg tgctgggtcg
cctgggcctg gaggtcggca agcgcatcga actggcaggc ggcaggcagg tgcagccata
catcaaggcc agcgtgttgc aggagttcga cggcgcgggt acggtacgca ccaacggcat
cgcgcatcgc accgaactgc gcggcacgcg cgccgaactg ggcctgggca tggccgccgc
gctgggccgc ggccacagcc tgtatgcctc gtacgagtac tccaagggcc cgaagctggc
catgccgtgg accttccacg cgggctaccg gtacagctgg taaagcgaga agggtccatc
ccccgcggag gagtttttcc tggaggttgg ccggtgccag tctccaggct caggcggcca
gggcctgcgg gccgggcagg ccgtgctggt gctggccgaa ccattgcaca gggtgttcgg
ccaagggcgg cgacttcgcc gatgaccagc aacgccgggg ggcgcacgct gcgccggcgc
gcgatc

** Region I

B. parapertussis p.70 protein [SEQ ID NO:6]

MNMSLSRIVK AAPLRRTTLA MALGALGAAP AAYADWNNQS IIKAGERQHG IHIKQSDGAG
VRTATGTTIK VSGRQAQGVL LENPAAELRF QNGSVTSSGQ LFDEGVRRFL GTVTVKAGKL
VADHATLANV SDTRDDDGIA LYVAGEQAQA SIADSTLQGA GGVRVERGAN VTVQRSTIVD
GGLHIGTLQP LQPEDLPPSR VVLGDTSVTA VPASGAPAAV FVFGANELTV DGGHITGGRA
AGVAAMDGAI VHL[QRATIRR GDAPAGGAVP GGAVPGGAVP GGFGPLLDGW YGVDVSDSTV
DLAQ]*SIVEAP QLGAAIRAGR GARVTVSGGS LSAPHGNVIE TGGGARRFPP
PASPLSITLQ AGARAQGRAL LYRVLPEPVK LTLAGGAQGQ GDIVATELPP IPGASSGPLD
VALASQARWT GATRAVDSLS IDNATWVMTD NSNVGALRLA SDGSVDFQQP AEAGRFKVLM
VDTLAGSGLF RMNVFADLGL SDKLVVMRDA SGQHRLWVRN SGSEPASGNT MLLVQTPRGS
AATFTLANKD GKVDIGTYRY RLAANGNGQW SLV[GAKAPPA PKPAPQPGPQ PGPQPPQPPQ
PPQPPQPPQP PQRQPEAPAP QPPAGRELSA A]**ANAAVNTGG VGLASTLWYA
ESNALSKRLG ELRLNPDAGG AWGRGFAQRQ QLDNRAGRRF DQKVAGFELG ADHAVAVAGG
RWHLGGLAGY TRGDRGFTGD GGGHTDSVHV GGYATYIANS GFYLDATLRA SRLENDFKVA
GSDGYAVKGK YRTHGVGVSL EAGRRFAHAD GWFLEPQAEL AVFRVGGGAY RAANGLRVRD
EGGSSVLGRL GLEVGKRIEL AGGRQVQPYI KASVLQEFDG AGTVRTNGIA HRTELRGTRA
ELGLGMAAAL GRGHSLYASY EYSKGPKLAM PWTFHAGYRY SW

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* Region I

** Region II

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What is claimed is:

1. An immunogenic composition comprising a mixture of pertactins of *Bordetella* species, wherein said composition comprises:

(a) pertactin of *Bordetella parapertussis*, and

(b) pertactin of *Bordetella bronchiseptica*,

in amounts sufficient to induce a humoral or cellular immune response against *Bordetella parapertussis* and *Bordetella bronchiseptica* in an animal to which the immunogenic composition is administered.

2. An immunogenic composition as claimed in claim 1, which also comprises pertactin of *Bordetella pertussis* in an amount sufficient to induce a humoral or cellular immune response against *Bordetella pertussis* in an animal to which the immunogenic composition is administered.

3. An immunogenic composition comprising a mixture of pertactins of *Bordetella* species or fragments thereof, wherein said pertactins or fragments thereof comprise at least two *Bordetella bronchiseptica* pertactin variants and wherein said pertactins of *Bordetella* species or fragments thereof are present in an amount sufficient to induce a humoral or immune response against *Bordetella bronchiseptica* in an animal to which the composition is administered.

4. An immunogenic composition according to claim 3, comprising a mixture of pertactins of *Bordetella* species or fragments thereof, wherein said pertactins or fragments thereof comprise at least two *Bordetella bronchiseptica* pertactin variants, and said at least two *Bordetella bronchiseptica* pertactin variants differ from each other at least in their Region II.

5. An immunogenic composition comprising a mixture of pertactins of *Bordetella* species or fragments thereof, wherein said pertactins or fragments thereof comprise a mixture of *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and wherein said *Bordetella bronchiseptica* pertactin variants are present in amounts sufficient to induce a humoral or cellular immune response against *Bordetella bronchiseptica* in an animal to which the immunogenic composition is administered.

6. An immunogenic composition according to claim 5, wherein at least two of the *Bordetella bronchiseptica* pertactin variants differ from each other at least in the number of repeating PQP amino acid sequences in their Region II.

7. An immunogenic composition as claimed in claim 5 or 6, which also comprises pertactins of *Bordetella parapertussis* or fragment thereof, *Bordetella pertussis* or fragment thereof, or mixtures thereof, in amounts sufficient to induce a humoral or cellular immune response against *Bordetella parapertussis* or *Bordetella pertussis* in an animal to which the immunogenic composition is administered.

8. An immunogenic composition according to claim 3, comprising a mixture of pertactins of *Bordetella* species or fragments thereof, wherein said pertactins or fragments thereof comprise at least two *Bordetella bronchiseptica* pertactin variants, and said at least two *Bordetella bronchiseptica* pertactin variants differ from each other at least in their Region I.

9. An immunogenic composition comprising a mixture of pertactins of *Bordetella* species or fragments thereof, wherein said pertactins or fragments thereof comprise a mixture of *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof, and wherein said *Bordetella bronchiseptica* pertactin variants are present in amounts sufficient to induce a humoral or cellular immune response against *Bordetella bronchiseptica* in an animal to which the immunogenic composition is administered.

10. An immunogenic composition according to claim 9, wherein at least two of the *Bordetella bronchiseptica* pertactin variants differ at least from the number of repeating GGXXP amino acid sequences in their Region I.

11. An immunogenic composition as claimed in claim 9, which also comprises pertactins of *Bordetella parapertussis* or a fragment thereof, *Bordetella pertussis* or a fragment thereof, or mixtures thereof, in amounts sufficient to induce a humoral or cellular immune response against *Bordetella parapertussis* or *Bordetella pertussis* in an animal to which the immunogenic composition is administered.

12. An immunogenic composition comprising a mixture of pertactins of *Bordetella bronchiseptica* species or fragments thereof, wherein said pertactins or fragments thereof comprise a mixture of *Bordetella bronchiseptica* pertactin variants, wherein at least one of said *Bordetella bronchiseptica* pertactin variants comprises Region II of pertactin of *Bordetella bronchiseptica* having 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and at least another of said *Bordetella bronchiseptica* pertactin variants comprises Region I of pertactin of *Bordetella bronchiseptica* having 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof.

13. An immunogenic composition comprising a mixture of fragments of pertactins of *Bordetella* species, wherein said composition comprises:

(a) pertactin of *Bordetella parapertussis* or a fragment thereof, and

(b) pertactin of *Bordetella bronchiseptica* or a fragment thereof containing Region I, Region II, or both Region I and Region II,

in amounts sufficient to induce a humoral or cellular immune response against *Bordetella parapertussis* and *Bordetella bronchiseptica* in an animal to which the immunogenic composition is administered.

14. An immunogenic composition as claimed in claim 13, which also comprises pertactin of *Bordetella pertussis* or a fragment thereof in an amount sufficient to induce a humoral or cellular immune response against *Bordetella pertussis* in an animal to which the composition is administered.

15. A composition comprising a mixture of at least two *Bordetella bronchiseptica* pertactin variants, wherein each variant comprises Region II of a pertactin of *Bordetella bronchiseptica*, and wherein said variants differ from each other at least in the Region II they each comprise.

16. A composition comprising a mixture of *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and at least two *Bordetella bronchiseptica* pertactin variants differ in the number of said repeating PQP amino acid sequences contained therein.

17. A composition as claimed in claim 16, which also comprises pertactins of *Bordetella parapertussis* or a fragment thereof, *Bordetella pertussis* or a fragment thereof, or mixtures thereof.

18. A composition comprising a mixture of at least two *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises Region I of pertactin of *Bordetella bronchiseptica*, and wherein said variants differ from each other at least in the Region I that each comprises.

19. A composition comprising a mixture of *Bordetella bronchiseptica* pertactin variants, wherein each *Bordetella bronchiseptica* pertactin variant comprises 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof, and at least two *Bordetella bronchiseptica* pertactin variants differ in the number of said repeating GGXXP amino acid sequences contained therein.

20. A composition as claimed in claim 19, which also comprises pertactins of *Bordetella parapertussis* or a fragment thereof, *Bordetella pertussis* or a fragment thereof, or mixtures thereof.

21. A composition comprising a mixture of *Bordetella bronchiseptica* pertactin variants, wherein one of said *Bordetella bronchiseptica* pertactin variants comprises Region II of pertactin of *Bordetella bronchiseptica* and another of said *Bordetella bronchiseptica* pertactin variants comprises Region I of pertactin of *Bordetella bronchiseptica*.

22. The composition according to claim 21, wherein said Region II has 6, 7, 8, or 9 repeating PQP amino acid sequences.

23. The composition according to claim 21, wherein said Region I has 1, 2, or 3 repeating GGXXP amino acid sequences.

24. A composition comprising a mixture of *Bordetella bronchiseptica* pertactin variants, wherein one of said *Bordetella bronchiseptica* pertactin variants comprises Region II of pertactin of *Bordetella bronchiseptica* having 6, 7, 8, or 9 repeating PQP amino acid sequences in Region II thereof, and another of said *Bordetella bronchiseptica* pertactin variants comprises Region I of pertactin of *Bordetella bronchiseptica* having 1, 2, or 3 repeating GGXXP amino acid sequences in Region I thereof.

25. A composition as claimed in claim 24, which also comprises pertactins of *Bordetella parapertussis* or a fragment thereof, *Bordetella pertussis* or a fragment thereof, or mixtures thereof.

26. A polypeptide comprising a sequence or a fragment of said sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22.

27. A polypeptide consisting of the amino acids in SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22.

28. A polynucleotide encoding a polypeptide as claimed in claim 26.

29. A purified DNA or RNA sequence that hybridizes under moderate or high stringency conditions to the polynucleotide of claim 28 or at least to 15 nucleotides thereof.

30. A polynucleotide encoding a polypeptide as claimed in claim 27.

31. A purified DNA or RNA sequence that hybridizes under moderate or high stringency conditions to the polynucleotide of claim 30 or at least to 15 nucleotides thereof.

32. Purified antibodies that bind to a polypeptide of claim 26.

33. Purified antibodies according to claim 32, wherein the antibodies are monoclonal antibodies.

34. Purified antibodies according to claim 32, wherein the antibodies are polyclonal antibodies.

35. An immunological complex comprising a polypeptide of claim 26 and an antibody that specifically recognizes said polypeptide.

36. A method for detecting infection by Bordetella, wherein the method comprises providing a composition comprising a biological material suspected of being infected with Bordetella, and assaying for the presence of a polypeptide of claim 26.

37. The method as claimed in claim 36, wherein the polypeptide is assayed by electrophoresis or by immunoassay with antibodies that are immunologically reactive with the polypeptide.

38. An *in vitro* diagnostic method for the detection of the presence or absence of antibodies, which bind to an antigen comprising a polypeptide of claim 26, wherein the

method comprises contacting the antigen with a biological fluid for a time and under conditions sufficient for the antigen and antibodies in the biological fluid to form an antigen-antibody complex, and detecting the formation of the complex.

39. The method as claimed in claim 38, which further comprises measuring the formation of the antigen-antibody complex.

40. The method as claimed in claim 38, wherein the formation of antigen-antibody complex is detected by immunoassay based on Western blot technique, ELISA, indirect immunofluorescence assay, or immunoprecipitation assay.

41. A diagnostic kit for the detection of the presence or absence of antibodies, which bind a polypeptide of claim 26 or mixtures thereof, wherein the kit comprises an antigen comprising polypeptide of claim 26 or mixtures of said polypeptides, and means for detecting the formation of immune complex between the antigen and antibodies, wherein the means are present in an amount sufficient to perform said detection.

42. An immunogenic composition comprising at least one polypeptide of claim 26 in an amount sufficient to induce an immunogenic or protective response *in vivo*, and a pharmaceutically acceptable carrier therefor.

43. The immunogenic composition as claimed in claims 1 to 14, wherein said composition comprises a neutralizing amount of at least one polypeptide of claim 26.

44. An immunogenic composition comprising a polynucleotide according to any one of claims 28 to 31.

45. A vaccine comprising an immunogenic composition according to any one of claims 1 to 14, 42, 43 or 44 along with a pharmaceutically acceptable vehicle.

46. A method for detecting the presence or absence of Bordetella comprising:

(1) contacting a sample suspected of containing genetic material of Bordetella with at least one nucleotide probe, and

(2) detecting hybridization between the nucleotide probe and the genetic material in the sample,

wherein said nucleotide probe is complementary to a polynucleotide sequence as claimed in any one of claims 28 or 30.

47. A vaccination kit comprising at least an immunogenic composition according to claims 1 to 14 or 42 to 44, and means for administering the composition to an animal.

48. An immunogenic composition consisting essentially of:

(A) a polypeptide comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin of *Bordetella pertussis*;

(B) a polypeptide comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin of *Bordetella parapertussis*;

(C) a polypeptide comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin of *Bordetella bronchiseptica* strain 9.73 and a polypeptide comprising Region I and Region II, or one polypeptide comprising Region I and one polypeptide comprising Region II, of a pertactin of *Bordetella bronchiseptica* of strain SEI.

49. An immunogenic composition consisting essentially of:

(A) a pertactin of *Bordetella bronchiseptica*;

(B) FHA of *Bordetella bronchiseptica*; and

(C) a pertactin of *Bordetella parapertussis*.

50. The immunogenic composition as claimed in claim 49, wherein the pertactin of *Bordetella bronchiseptica* is from strain 9.73.

51. The immunogenic composition as claimed in claim 49, wherein the FHA of *Bordetella bronchiseptica* is from strain 9.73.

52. An immunogenic composition as claimed in any one of claims 1 to 14, 42 to 44, or 48 to 51, wherein the composition further comprises at least one adhesin of *Bordetella* selected from the group consisting of FHA, AGG2, AGG3, and/or at least one toxin of *Bordetella* selected from the group consisting of PTX, DNT, TCT, Ac-Hly.

53. A DNA chip, wherein said chip comprises at least one polynucleotide according to claims 28 to 31 or fragment thereof.

54. Use of monoclonal antibodies according to claim 33 for treating *Bordetella* infections.

55. A microarray comprising microbeads, wherein said microbeads each bears multiple copies of a polynucleotide according to any one of claims 28 to 31 or a fragment

thereof, and wherein the polynucleotide or fragment thereof is different from one microbead to another.

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218 Fig. 1a: The two regions of repeats in *Bordetella bronchiseptica* pertactin

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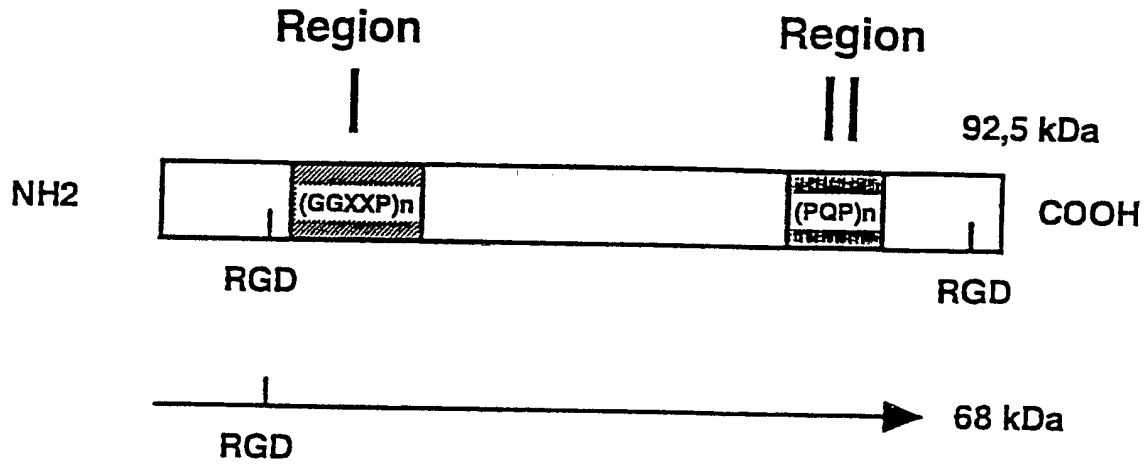

Fig. 1b: Multiple alignment of *B. bronchiseptica* pertactin in region I

I-1	QRATIRRGDAPAGGAVPGGAVPGGAVPG----------GFGPLLDGWYGVDVSDSTVDLAQ (SEQ ID NO: 7)
I-2	QRATIRRGDAPAGGAVPG-----GAVPG----------GFGPLLDGWYGVDVSDSTVDLAQ (SEQ ID NO: 8)
I-3	QRATIRRGDAPAGGGVPG-----GAVPG-----GFDPGGFGPGGFGPVLDGWYGVDVSGSTVELAQ (SEQ ID NO: 9)
prn1	QRATIRRGDAPAGGAVPG-----GAVPG-----GAVPGGFGPGGFGPVLDGWYGVDVSGSSVELAQ (SEQ ID NO: 10)
prn2	QRATIRRGDAPAGGAVPG-----GAVPGGFGPGGFGPGGFGPGGFGPVLDGWYGVDVSGSSVELAQ (SEQ ID NO: 11)
prn3	QRATIRRGDAPAGGAVPG-----GAVPG-----GFGPGGFGPGGFGPVLDGWYGVDVSGSSVELAQ (SEQ ID NO: 12)
prn4	QRATIRRGDAPAGGAVPG-----GAVPG----------GFGPGGFGPVLDGWYGVDVSGSSVELAQ (SEQ ID NO: 13)
	********** *** ***** ****.********** *.*.***

Fig. 1c: Multiple alignment of *B. bronchiseptica* pertactin in region II

II-1	GAKAPPAPKPAPQPGPQPGP----------QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 14)
II-2	GAKAPPAPKPAPQPGPQPGPQPP--------QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 15)
II-3	GAKAPPAPKPAPQPGPQPGPQPGPQPGPQPPQPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 16)
II-4	GAKAPPAPKPAPQPGPQPGPQPGP--------QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 17)
II-5	GAKAPPAPKPAPQPGPQPGPQPGPQP----PQPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 18)
II-6	GAKAPPAPKPAPQPGPQPGPQPPQPP--QPPQPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 19)
II-7	GAKAPPAPKPAPQPGPQP-P----------QPPQPPQP-PQRQP--EAPAPQPPAGRELSAA (SEQ ID NO: 20)
II-8	GAKVPPAPKPAPQPGPQP-PQPP--------QPPQPPQPQPQPQP--EAPAPQPPAGRELSAA (SEQ ID NO: 21)
II-9	GAKVPPAPKPAPQPGPQP-PQPP--------QPPQPPQPQPQPQPQPEAPAPQPPAGRELSAA (SEQ ID NO: 22)
prnl	GAKAPPAPKPAPQPGPQP----------PQPPQP----QP--EAPAPQPPAGRELSAA (SEQ ID NO: 23)
prn6	GAKAPPAPKPAPQPGPQP----------PQP----QP--EAPAPQPPAGRELSAA (SEQ ID NO: 24)