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The objective of this work is to implement a diagnostic protocol for Bordetella spp. by the semi-nested Polymerase Chain Reaction (sn-PCR)by means the detection of the gene which encodes the structural protein flagellin. For this, three in silico primers were designed using the free access Oligo Perfect ™ program, which was later synthesized by the BIOSCAN company.

Thus, using three strains of *B. bronchiseptica* as samples, fragments of the expected sizes were obtained (362 bp and 170 bp) and, after the sequencing of the larger amplicon, a percentage of 95% nucleotide identity was determined by the Clustal Ω program with respect to the official data registered in GenBank®. This value was corroborated when entering the same sequence to the BLAST online program, which also delivered 95% nucleotide identity percentage concerning to the flagellin protein gene of Bordetella spp.

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I. Introduction

B. bronchiseptica is a Gram-negative, strict aerobic coccobacillus. This microorganism can be found in the respiratory tract of dogs andother species such as cats, pigs, laboratory animals and humans. Its transmission is produced by direct contact, by the generation of contaminated aerosols or fomites. There is evidence of occasional transmission between species, particularly between dogs and cats it is associate with respiratory symptoms, where its role as the primary or secondary agent is still discussed(Molina *et al*., 2006). This is because there is evidence that this bacterium is involved in the presentation of acute cases of respiratory diseases, as well as that it is an etiologic agent secondary to a viral infection (Molina*et al*., 2006).

B. bronchiseptica is considered the etiological agent of canine infectious tracheobronchitis (CIT), known as "a kennel cough". Also, that canine parainfluenza virus, adenovirus type-2, canine herpesvirus, reovirus, fungi and *Mycoplasma sp*also participate in this scenario(Keil and Fenwick 2000).

In most cases, the infection remains localized in the respiratory tract. The congregation of dogs of different ages and different levels of susceptibility to the agents involved in the status predisposes to the presentation of the same. Because, the canine respiratory complex is a disease to prevent in human societies, veterinary hospitals, research institutes and places where exhibition dogs are housed or shown (Keil and Fenwick, 2000).

The ability to colonize ciliated epithelial cells of the respiratory tract is recognized in bacteria. This is due to the presence of fimbrial and non-fimbrial adhesins. Within the latter are hemagglutinin and pertectin, located mainly in the outer membrane of the bacteria. *B. bronchiseptica* produces endo and exotoxins, within the latter, a hemolysin adenylate cyclase, dermonecrotic toxin and tracheal cytotoxin. These factors affect the hair cells, inhibit the response of phagocytic cells, suppress the immune response and are associated with clinical signs in dogs with CIT (Keil and Fenwick, 2000).

In general, CIT is self-limiting and does not require antibiotic therapy. However, its prophylactic use is recommended in individuals who have been in contact with dogs that have presented the status (Keil and Fenwick, 2000).

Respiratory symptoms due to this bacterium occur in laboratory animals. The manifestations can vary from nasal discharge, sneezing, anorexia, and weight loss to bronchopneumonia and septicemia. Specifically, in rabbits it is known as the rabbit's catarrh (Matto and Cherry, 2005).

In humans, *B. bronchiseptica* is hardly found. However, there are cases of immunologically compromised people where this bacterium can cause upper respiratory tract infections, pneumonitis, endocarditis, peritonitis, meningitis and bacteremias. In humans with AIDS, this bacterium can cause interstitial pneumonia (Galeziok *et al*., 2009).

It is usually diagnosed clinically by symptomatology and the presence of predisposing factors, such as environments of high animal load such as exposures, breeding sites, hotels, hospitals or another group situation (Keil and Fenwick 2000).

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Regarding the microbiological diagnosis, the samples may come from nasal swabs or transtracheal lavage. The tracheal samples are privileged, because the problem that can identify the *B. bronchiseptica* that is part of the normal microbiota of the oropharynx is eliminated (Keil and Fenwick 2000).

Although the bacteriological culture has a great diagnostic specificity, its sensitivity could be low when compared with molecular diagnostic techniques such as the PCR, which can give positive results despite the antibiotic therapy, it also allows to investigate samples with low counts of microorganisms and not necessarily viable (Osses *et al.* 2010). On the other hand, PCR is not limited by the ability of microorganisms to grow in culture media, in addition the result obtained can provide much more information than just indicating the presence of the bacteria, since it can provide usable data to determine epidemiological characteristics and phylogenetic (Couthino *et al.* 2009). In this regard, several PCR protocols have been established to identify different regions of the genome of several *Bordetella* species. However, most of them are focused on *Bordetella pertussis (B. pertussis)*, a microorganism that causes pertussis in humans (Couthino *et al.* 2009, Tatti *et al.*2011, Grogan *et al.*2011, Lanotte *et al*. 2011; Tatti and Tondella 2013).

Thus, in this work the molecular diagnosis of *Bordetella spp*. by the sn-PCR using the design of *in silico*primers to identify the *fla*A gene is already implemented. The gene that encode this protein are ideal candidates to act as biomarkers, since being a protein located on the surface of the bacterium show a much higher divergence rate in their amino acid sequence than those located inside the cell (Winstanley *et al.,* 1997). It has also been shown that the flagellum is necessary to initiate and potentiate the interaction between the bacterium and the cell surface, which is why the flagellum has the potential to distinguish between several host species (Nicholson *et al* 2012).

II. Materials and Methods

Samples. Three strains of *B. bronchiseptica* are used, two from the Institute of Public Health of Chile (ISP): (BB1, BB2) and another from the Microbiology Laboratory of FAVET: (BB3). DNA from *Salmonella* Enteritidis (SE) and from Canine Herpes Virus (CaHV-1) was used as a negative control. Nuclease-free water (NFW) was used as reagent control.

Bacterial DNA obtaining. The extraction of bacterial DNA was carried out using a commercial kit (Genomic DNA Purification kit, Fermentas®). From cultures of $8x10^6$ CFU / mL -obtained by turbidity compared to tube 0.5 of McFarland- 200 µL were taken, to which 400 µL of lysis solution was added, being incubated for five minutes at 65° C. After that time, 600. µL of chloroform were added, mixing gently and then centrifuged at 10,000 rpm for two minutes (Heraeus SepatechBiofuge®). Subsequently, the upper phase was collected in an Eppendorf tube and 800 µL of precipitation solution was added, to re-centrifuge at 10,000 rpm for two minutes. The supernatant was removed and the DNA was resuspended with 100 µL of 1.2 M Sodium Chloride solution and vortexed. To this mixture was added 300 µL of cold ethanol and then kept at -20 ° C for ten minutes. It was centrifuged again at 10,000 rpm, for three minutes, the supernatant was removed and resuspended in 100 µL of nuclease-free water (Winkler®), thus obtaining the DNA that was used in the PCR.

Detection of the *fla*A gene by the semi-nested polymerase chain reaction. Primers: Using the Oligo Perfect ™ program, two pairs of primers were designed whose target sequence is the *fla*A gene (Table 1, Table 2). Then they were sent to synthesize by means the company BIOSCAN. The sequence of the synthesized primers is as follows:

BOR 1F	ACCTGAACAAGTCCCAATCG
BOR 1R= BOR 2R	GACCTTGATGCCGTTGAAGT
BOR 2F	CGCTGAACGAAATCAACAAC

As you can see, only three primers are designed. It is expected that BOR 1F and BOR 1R generate an amplicon of 362 base pairs (Annex 2a), while BOR 2F and BOR 2R generate an amplicon of 170 base pairs (Annex 2b). For this, strictly speaking, this is a semi-nested PCR, since the BOR 1R (or BOR 2R) participates in both reactions.

Reaction mixture: 15 µL of the commercial Master Mix 2X PCR kit (Taq DNA polymerase, $MgCl_2$ and the deoxyribonucleotid triphosphates), fiveµL of template DNA and fiveµL of each specific primer were used, reaching a final volume of 30 µL.

DNA amplification: The PCR technique contemplated a stage of DNA denaturation, followed by a stage of alignment of the primers and a final stage of elongation. The semi-nested variant consisted of using a second pair of primers that amplified a fragment of smaller size since it used the amplicon generated by the first pair of primers as the template. The determination of the alignment temperatures for each pair of primers considered the use of a temperature gradient thermal cycler following a standard PCR scheme: initial denaturation at 94°C (two minutes), then 35 cycles: denaturation (94°C, one minute), alignment, elongation (72°C, one minute) and finally an elongation period for five minutes at 72 ° C.

Visualization of the amplified product: It was carried out by electrophoresis in 2% agarose gel (Winkler ®) in Tris-borate buffer (90 Mm Tris-borate, 10 mM EDTA) as solvent. An aliquot of 6 µL of this mixture was deposited in the corresponding well in the gel. Electrophoresis was

carried out at 90 volts for 40 minutes. As a molecular size marker, a standard containing DNA fragments between 100 and 1000 base pairs (bp) (Hyperladder IV, Bioline®) was used. After electrophoresis, the gel was incubated in Ethidium Bromide (0.5 µg / mL) (Fermelo ®) for 35 minutes and then placed in an ultraviolet transilluminator (Transiluminator UVP ®), where it was finally photographed. to obtain record of the results.

Determination of the percentage of nucleotide identity with respect to GenBank®. Five DNA fragments obtained by PCR1 were sent to the company Genytec for the determination of their nucleotide sequence.

Analysis. To obtain a consensus sequence, the 5 sequences obtained were aligned using the Clustal Ω program. The consensus sequence was aligned with one of the official data of the Genbank (access number L13034.1) to obtain the nucleotide identity percentage, which was corroborated using the BLAST *online* program.

Biosafety measures. For this work, the necessary biosafety measures are associated with the manipulation of the *B. bronchiseptica* bacteria, the performance of the PCR and the subsequent visualization of the amplified product. Regarding the former, limited access to facilities was considered, the use of Bunsen burner to delimit a sterile work area, the use of an apron, the use of clean material and the proper disposal of waste. Regarding the implementation of the PCR, a clean and exclusive area for this procedure was delimited, to avoid contamination with genetic material not coming from the sample, and latex gloves were used to carry out the procedures, such as, for example, the use of ethidium bromide, which has mutagenic properties. At the time of using the ultraviolet light transilluminator they were used with UV filter goggles and an acrylic plate placed between the equipment and the one who visualizes the gel. Finally, the gel was incinerated along with the gloves that were used for its handling.

III. Results

Detection of the *fla*A gene by the sn-PCR. Figure 1 shows an Agarose gel subjected to electrophoresis (90 volt, 40 minutes) and incubated in Ethidium Bromide (35 minutes). It displays fragments of DNA of approximate sizes to those expected (362 and 170 bp) by having the size marker between 100 and 1000 bp as reference. The fluorescent bands are clear and unique. No bands are observed in the lanes of the negative or reagent controls.

Determination of the percentage of nucleotide identity with respect to GenBank®. The DNA fragments obtained by PCR for the sample BB3 were sent in fivefold to the company Genytec for the determination of its nucleotide sequence, receiving 5 sequences (Table 3) and obtaining the following consensus sequence from them:

>NTV6
TGAACAAGTTCCAATCGGCCCTGTGTAGCGCCATCGA
CCGCCTGTCGTCGGGTCTGAGCATCAACAGCGCCAA
GGACGACGCGGCCGTCCAGGCCATCGCCTACCGCT
TCACCGCCAACGTCAAGGGCCTGACCAAGGCTGCCC
GCAACGCCAACGACGGCATCTTGATCGCCCAGACGT
CCGAAGGCGCGCTCAACGAAATCAAGAACAACATGC
AGCGCATCCGCGAACTGACAGTTCAGGTCTCCAACG
GCACGTACTCGGCTTCGGACATCGACTCGATCCAGC
AGAAAGTCAACCAGCGCCTGGAAGAAATCAACCCATC
GCCGAGCAGTCCGACTTCAACGGCA

The alignment of this consensus sequence versus the official GenBank data (access number L13034.1) yielded a NIP value of 95% (Table 4).

This NIP value was confirmed in parallel with the value delivered by the BLAST program, which also delivered a value of 95% (Table 5).

IV. Discussion

The diagnosis of *B. bronchiseptica* through bacterial culture involves an incubation period at 37 ° C for 48 hours (Keil and Fenwick, 2000, Couthino *et al* 2009). This time is reduced by using a molecular diagnostic technique such as PCR. However, it is not only in terms of time that PCR represents an advantage over culture, since in terms of sensitivity the PCR technique has yielded positive results in suspicious samples that, when diagnosed by bacteriological culture, have delivered negative results (Canonne *et al*., 2016)

Additionally, and given the range of respiratory charts in which *B. bronchiseptica* participates, implementing a PCR protocol for the diagnosis of this agent is necessary. In fact, during this report, the primers and the proposed protocol were used by professionals of the Microbiology Laboratory to identify microorganisms suspected of corresponding to *B. brochiseptica,* demonstrating the usefulness of the work carried out.

The decision to perform a semi-nested PCR responded to the advantages of this variant over conventional PCR. Although a PCR with only a pair of primers can perfectly serve as an accurate diagnostic method, a nested-PCR increases both the specificity and sensitivity (Mendoza *et al.,* 2001). In fact, the PCR performed corresponds to the semi-nested variant because it was decided to use the primers that the online program suggested as "the best" against the sequence to be identified. In this case the BOR 1R (or BOR 2R) participates in the first reaction with the BOR 1F, and then in the second reaction with the BOR 2F.

Related to the above, after the experience that allowed demonstrating the usefulness of the primers and the protocol, because reactions were performed with other samples suspected of containing *B. bronchiseptica* obtaining positive results. However, what is interesting is that what was described in the preceding lines was observed in practice: there were samples that when confronted with a pair of primers (conventional PCR) gave a negative result, but when the semi-nested PCR was carried out, they turned out to be positive This can be due to an error in the procedure (the non-incorporation of a primer, for example), or to an effect of the number of reaction cycles, since a quantity of DNA not visualized with the first PCR, certainly can be after the second reaction.

The nucleotide identity of the PCR amplicons would correspond to the *fla*A gene of *Bordetella spp.*, according to the of nucleotide identity percentage (NIP = 95%) obtained using the Clustal Ω program (comparing the consensus sequence with one of the data Genbank) or the incorporation of the consensus sequence in the BLAST Program. The above would be validated by obtaining a NIP value≥ 80% (Chopra and Roberts 2001)

Given these results and the extra experiences carried out, it is still pending to continue testing these primers and the protocol in terms of sensitivity. Perhaps making dilutions of a sample and determining to what extent this protocol continues to give a positive result is an experience that could be retaken from here.

The experience of choosing a bacterial agent, investigating its characteristics and investigating what has already been done to generate a diagnosis, is certainly useful to contemplate the theoretical and practical framework of diagnostic work in a laboratory.

Designing primers using the available technological tools and using them in a real experience constitutes a "downhill" enough to know in what terrain the veterinarian themes moves in the diagnostic area today. Having designed a "own method", but that has given more expected results allowing us to identify the proposed agent, is very enriching both as an academic exercise, but also as a concrete tool that, as already mentioned, was already used in an opportunity in the laboratory. In this way, a PCR protocol has been implemented in the Microbiology Laboratory of the Department of Preventive Animal Medicine, in its semi-nested variant that can be used both for the diagnosis of suspicious samples and for future memories of the title of other students.

V. Conclusions

The designed primers allowed to originate unique and clear DNA bands of the expected size in both PCR1 (362 bp) and PCR 2 (170 PB). The presence of these fragments suggests, although it does not guarantee, the presence of the *fla*A gene in the strains. For this reason, nucleotide sequencing is necessary, a procedure that allows us to affirm that the fragment synthesized corresponds to the gene in question. Thus, using the Clustal Ω program, the alignment of the sequenced fragment (consensus sequence obtained from the sample sent in quintuplicate) compared to the sequence provided by GenBank yielded a nucleotide identity percentage of 95.16%. In addition, through the BLAST program, a 95% nucleotide identity percentage was obtained in relation to the flagellin gene, both *B. bronchiseptica* and *B. pertussis.*

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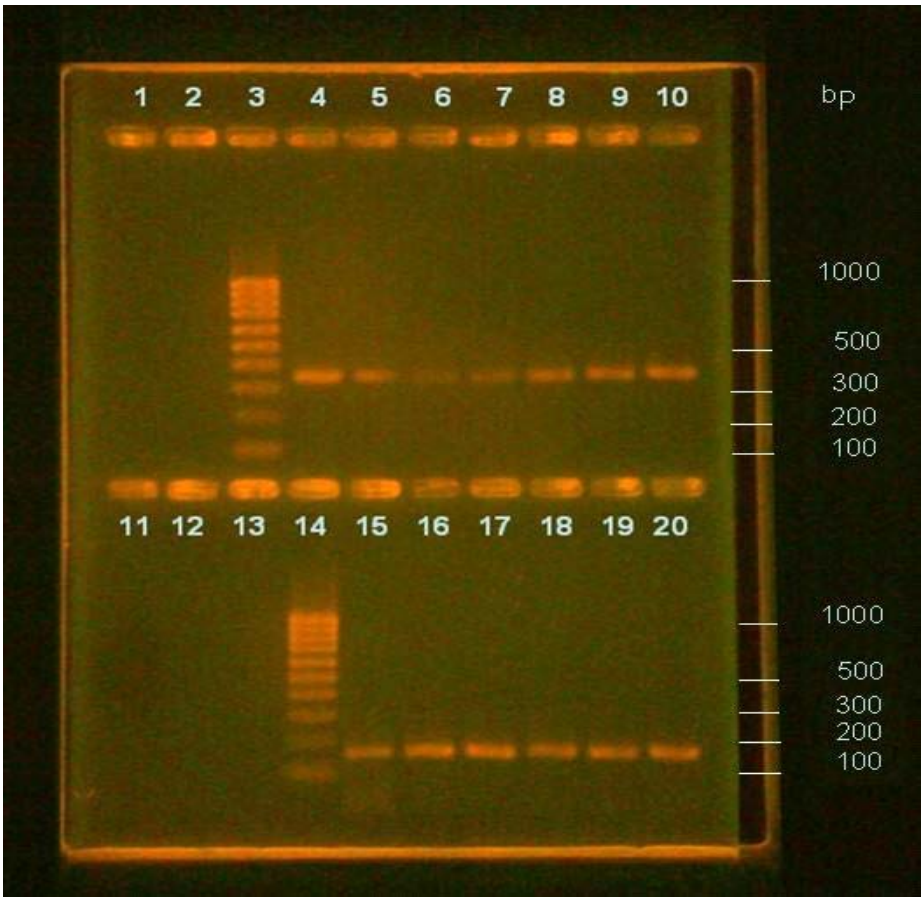


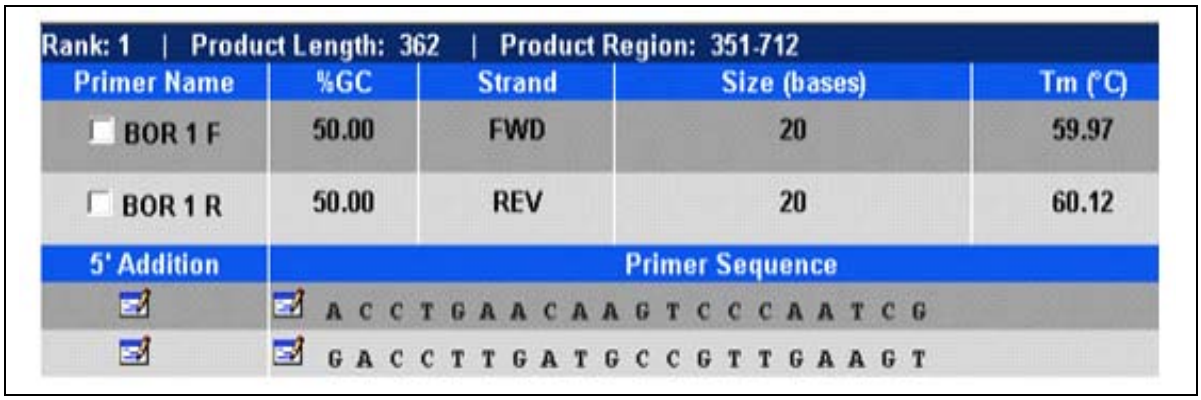
Figure 1: 2% agarose gel electrophoresis

PCR1. Lanes 1, 2: Negative control; lane 3: Marker; lanes 4, 5, 6: DNA from BB1; lanes 7, 8, 9: DNA from BB2; lane 10: DNA from BB3.

PCR2. Lanes 11,12: Negative control; Lane 13: reagent control; Lane 14: Marker; Lane 15,16: DNA from BB1: Lanes17,18: DNA from BB2; Lanes 19,20: DNA from BB3.

Negative control: DNA from *Salmonella* Enteritidis and canine herpes virus. Reagent control: nuclease-free water. MTM: Molecular size marker. 100-1000 bp (Hyperladder IV, Bioline®)

Table 1: Design of **BOR 1F** and **BOR 1R** primers by using Oligoperfect™ *online* program

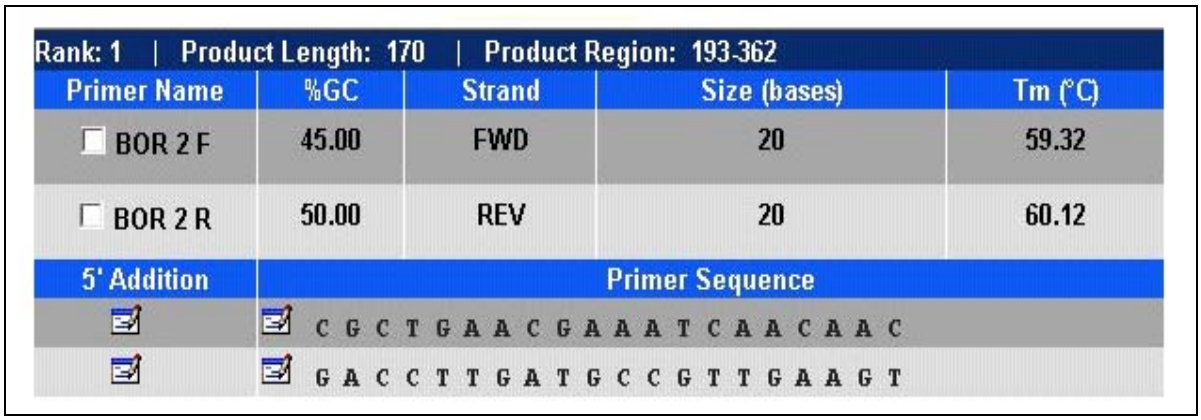


Rank: 1 | Product Length: 362 | Product Region: 351-712

Primer Name	%GC	Strand	Size (bases)	Tm (°C)
BOR 1 F	50.00	FWD	20	59.97
BOR 1 R	50.00	REV	20	60.12

5' Addition	Primer Sequence
	A C C T G A A C A A G T C C C A A T C G
	G A C C T T G A T G C C G T T G A A G T

Table 2: Design of **BOR2F** and **BOR2R** primers by using Oligoperfect™ online program



Rank: 1 | Product Length: 170 | Product Region: 193-362

Primer Name	%GC	Strand	Size (bases)	Tm (°C)
BOR 2 F	45.00	FWD	20	59.32
BOR 2 R	50.00	REV	20	60.12

5' Addition	Primer Sequence
	C G C T G A A C G A A A T C A A C A A C
	G A C C T T G A T G C C G T T G A A G T

Table 3: Obtaining consensus sequence according to Clustal Ω

```
NTV2 ------TGAAAAGTTCCAATCGGCCCTGTGTAGCGCCATCGACCGCCTGTCGTCGGGTTC  54
NTV3 ------TGAACAAGTTCCAATCGGCCCTGTGTAGCGCCATCGACCGCCTGTCGTCGGTTC  54
NTV1 ---------ACAAGTTCCAATCGGCCCTGTGTAGCGCCATCGACCGCCTGTCGTCGGGTC  51
NTV4 -----CTGAACAAGTTCCAATCGACCCTGTGTAGCGCCATCGACCGCCTGTGGTCGGGTC  55
NTV5 TGCACCTGAACAAGTCCCAATCGGCCCTGAGTAGCGCCATCGGGCGCCTGTCGTCGGGTC  60
* **** ******* ***** ************  ******* ***** **

NTV2 TGAGGATCAACAGCGCCAAGGACGACGCGACCGTCCAGGCCATCGCCTACCGCTTCACCG 114
NTV3 TGAGCATCAACAGCGCCAAGGACGACGCGGCCGTACAGGCCATCGCCTACCGCTTCACCG 114
NTV1 TGAGCATCAAGAGCGCCAAGGACAACGCGGCCGTCCAGGCCATCGCCTACCGCTTCACCG 111
NTV4 TGAGCATCAACAGCGCCAAGGACGACGCGGCCGTCCAGGCCAACGCCAACCGCTTCACCG 115
NTV5 TGCGCATCAACAGCGCCAAGGACGACGCGGCCGGCCAGGCCATCGCCAAACGCTTCACGG 120
** * ***** ************ ***** ***  ******* **** * ******** *

NTV2 CCAACGTCAAGGGCCTGACCGAGGCTGCCCGCAACGCCAACGACGGCAACTTGATCGCCC 174
NTV3 CCAACGTCAAGGGCGTGACCAAGGCTGCCCGCAACGCCAACGACGGCATCTTGAACGCCC 174
NTV1 CCAACGTCAAGGGCCTGACCAAGGCTGGCCGCAACGCCAACGACGGCATCTTGATCGCCC 171
NTV4 CCAACGTGAAGGGCCTGACCAAGGCTGCCCGCAACGCCAACGACGGCATCTTGATCGCCC 175
NTV5 CCAACGTCAAGGGCCTGACCCAGGCTGCCCGCAACGCCAACGACGGCATCTCGATCGCCC 180
******** ****** ***** ****** ********************** ** ** *****

NTV2 AGACGTCCGAAGGCGCGCTCAACGAAATCAACAACAAGATGCAGCGCATCCGCGAACTGA 234
NTV3 AGACGTCCGAAGGCGCGCTCAACGAAATCAAGAACAACATGCAGCGCATCCGCGAACTGA 234
NTV1 AGACGTCCGAAGGCGCGCTCAACGAAATCAACAACAACATGCAGGGCATCCGCGAACTGA 231
NTV4 AAACGACCGAAGGCGCGCTCAACGGAATCAACAACAACATGCAGCGCATCCGCGAACTGA 235
NTV5 AGACGACCAAAGGCGGGCTGAACGAAATCAACAACAACCTGCAGCGCATCCGCGAACTGA 240
   ***** ** ****** *** **** ****** *****  ***** ****************
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NTV2 CGGTTCAAGTCTCCAACGGCACGTACTCGGCTTCGGACATCGACTCGATCCAGGAGAAAG294
NTV3 CGGTTCAGGTCTCAAACGGCACGTACTCGGCTTCGGACATCGACTCGAGCCAGCAGAAAG 294
NTV1 CAGTTCAGGTCTCCAACGGCACGTACTCGGCTTCGGACATCGACTCGATCCAGCAGAAAG 291
NTV4 CGGTTCAGGTCTCCAACGGCAAGAACTCGGCTTCGGACATGGACTCGATCCAGCAGAAAG 295
NTV5 CGGTTCAGGCCTCCAACGGCACGAACTAGGCTGCGGACATCGACTCGATCCAGCAGGAAG 300
* ***** * *** ******* * *** **** ******* ******* **** ** ***

NTV2 TCAACCAGCGCCTGGAAGAAATCAACATCATCGCCGAGCAGTCCGACTTCAACGGCA-TC 353
NTV3 TCAACCAGCGCCTGGAAGAAATCAACCTCATCACCGAGCAGTCCGACTTCAACGGCA-TC 353
NTV1 TGAACCAGCGCCTGGAAGAAATCAACCTCATCGCCGAGCAGTCCGACTTCAACGGCA-TC 350
NTV4 TCAACCAGCGCCTGGAAGAAATCAACCTCATCGCCGAGCAAACCGACTTCAACGGCAGTC 355
NTV5 TCAACCAGCGCCTGGAAGAAATCAACCGCATCGCCGAGCAGACCGAATTGAGCT------ 354
* ********************** **** ******* **** ** * *
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Table 4: Alignment of the 351 bp fragment (NTV6) compared to the official sequence of GenBank

```
L13034.1 CATGGCTGCAGTCATCAATACCAACTACTTGTCGCTGGTTGCCCAGAACAACCTGAACAA
NTV6     ----------------------------------------------------TGAACAA
                                                               *******

L13034.1 GTCCCAATCGGCCCTGGGTAGCGCCATCGAGCGCCTGTCGTCGGGTCTGCGCATCAACAG
NTV6     GTTCCAATCGGCCCTGTGTAGCGCCATCGACCGCCTGTCGTCGGGTCTGAGCATCAACAG
         ** ************* ************* ****************** **********

L13034.1 CGCCAAGGACGACGCGGCCGGCCAGGCCATCGCCAACCGCTTCACCGCCAACGTCAAGGG
NTV6     CGCCAAGGACGACGCGGCCGTCCAGGCCATCGCCTACCGCTTCACCGCCAACGTCAAGGG
         ******************** ************* ************************

L13034.1 CCTGACCCAGGCTGCCCGCAACGCCAACGACGGCATCTCGATCGCCCAGACGACCGAAGG
NTV6     CCTGACCAAGGCTGCCCGCAACGCCAACGACGGCATCTTGATCGCCCAGACGTCCGAAGG
         ******* ****************************** ************* *******

L13034.1 CGCGCTGAACGAAATCAACAACAACCTGCAGCGCATCCGCGAACTGACGGTTCAGGCCTC
NTV6     CGCGCTCAACGAAATCAAGAACAACATGCAGCGCATCCGCGAACTGACAGTTCAGGTCTC
         ****** *********** ****** ********************** ******* ***

L13034.1 CAACGGCACGAACTCGGCTTCGGACATCGACTCGATCCAGCAGGAAGTCAACCAGCGCCT
NTV6     CAACGGCACGTACTCGGCTTCGGACATCGACTCGATCCAGCAGAAAGTCAACCAGCGCCT
         ********** ********************************** ***************

L13034.1 GGAAGAAATCAACCGCATCGCCGAGCAGACCGACTTCAACGGCATCAAGGTCCTGAAGTC
NTV6     GGAAGAAATCAACCCATCCGCCGAGCAGTCCGACTTCAACGGCA----------------
         **************    ********** ***************
```

Nucleotide Identity Percentaje (NIP).

Seq A	Name	Length	Seq B	Name	Length	Score
1	NTV6	351	2	L13041	1572	95.16

Table 5: Consensus sequence submitted to the BLAST *online* program

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Bordetella bronchiseptica 253 complete genome	549	549	100%	6e-153	95%	HE965806.1
Bordetella bronchiseptica MO149 complete genome	549	549	100%	6e-153	95%	HE965807.1
Bordetella pertussis 18323 complete genome	549	549	100%	6e-153	95%	HE965805.1
Bordetella pertussis CS, complete genome	549	549	100%	6e-153	95%	CP002695.1
Bordetella bronchiseptica strain RB50, complete genome; segment 8/16	549	549	100%	6e-153	95%	BX640444.1
Bordetella parapertussis strain 12822, complete genome; segment 5/14	549	549	100%	6e-153	95%	BX640427.1
Bordetella pertussis strain Tohama I, complete genome; segment 3/12	549	549	100%	6e-153	95%	BX640413.1
Bordetella bronchiseptica flagellin (flaA) gene, complete cds	549	549	100%	6e-153	95%	L13034.1
Bordetella parapertussis Bpp5 complete genome	538	538	100%	1e-149	94%	HE965803.1
Bordetella bronchiseptica strain SB283 flagellin gene, partial cds	536	536	97%	4e-149	95%	AF232941.1
Bordetella bronchiseptica strain SB22 flagellin gene, partial cds	536	536	97%	4e-149	95%	AF232939.1
Bordetella bronchiseptica strain SB521 flagellin gene, partial cds	525	525	97%	9e-146	94%	AF232940.1
Thiobacillus denitrificans ATCC 25259, complete genome	111	111	55%	3e-21	77%	CP000116.1
Chromobacterium violaceum ATCC 12472, complete genome	73.1	73.1	60%	1e-09	73%	AE016825.1