

蓝舌病毒VP2蛋白与NS4蛋白 的生物信息学分析

13组

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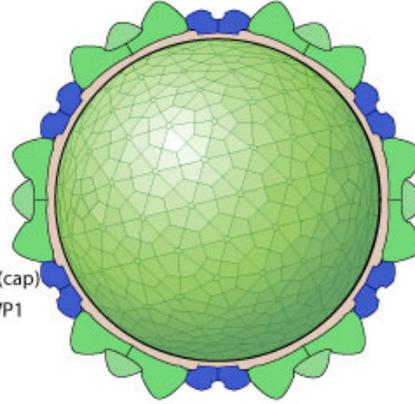
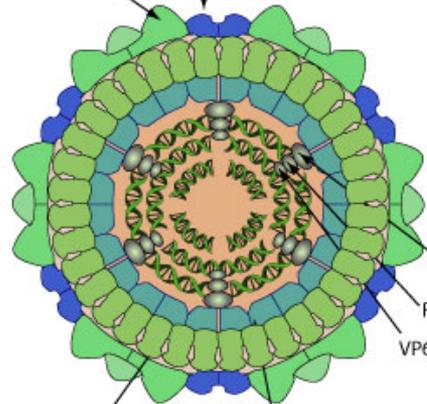
蓝舌病

- 蓝舌病(**Bluetongue**)是由呼肠孤病毒科环状病毒属蓝舌病病毒(**Bluetongue virus, BTV**)引起的反刍动物虫媒传染病。**BTV**能感染大多数家养的和野生的反刍动物，因动物的易感性不同表现出不同的临床症状。

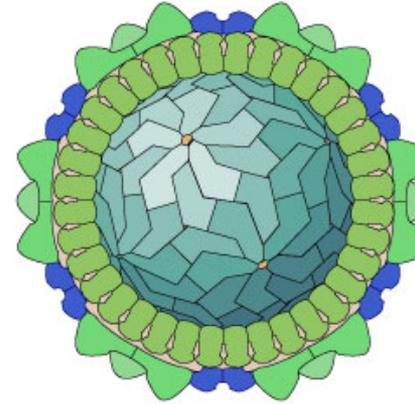
- 不同流行地区易感动物的死亡率差别很大，一般都可达到**30%**，在某些地区甚至更高。**BT**血清型众多，目前已发现**24**个血清型，最近又相继分离到**BTV25**和**26**型。自**2006**年**BTV8**在欧洲许多国家爆发，造成了严重的经济损失。我国目前还未有**BTV8**流行的相关报道，但是随着国际贸易的往来，**BTV8**随时有可能传入我国。

VIRION

OUTER CAPSID
VP2 trimer VP5 trimer



T=13
CORE CAPSID



T=2
INNER CAPSID

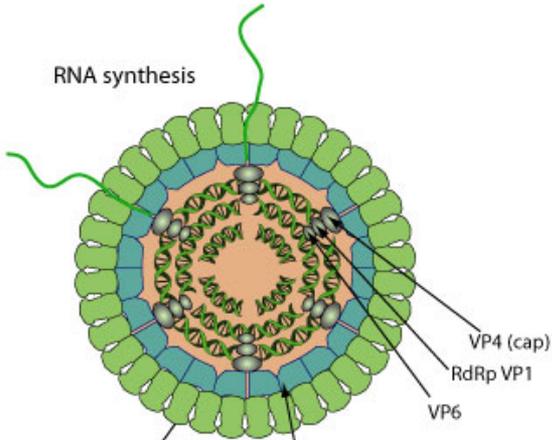
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VP7
CORE CAPSID VP3
INNER CAPSID

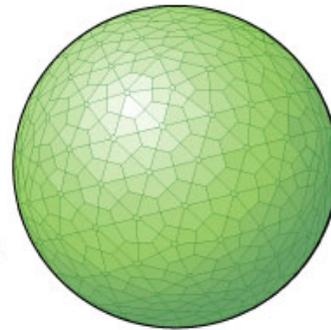


CORE PARTICLE

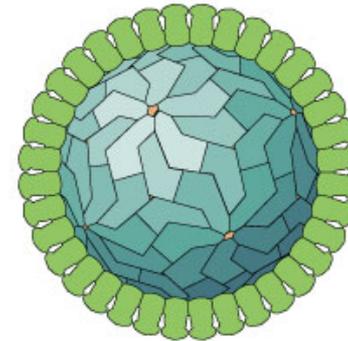
RNA synthesis



VP7
CORE CAPSID VP3
INNER CAPSID



T=13
CORE CAPSID



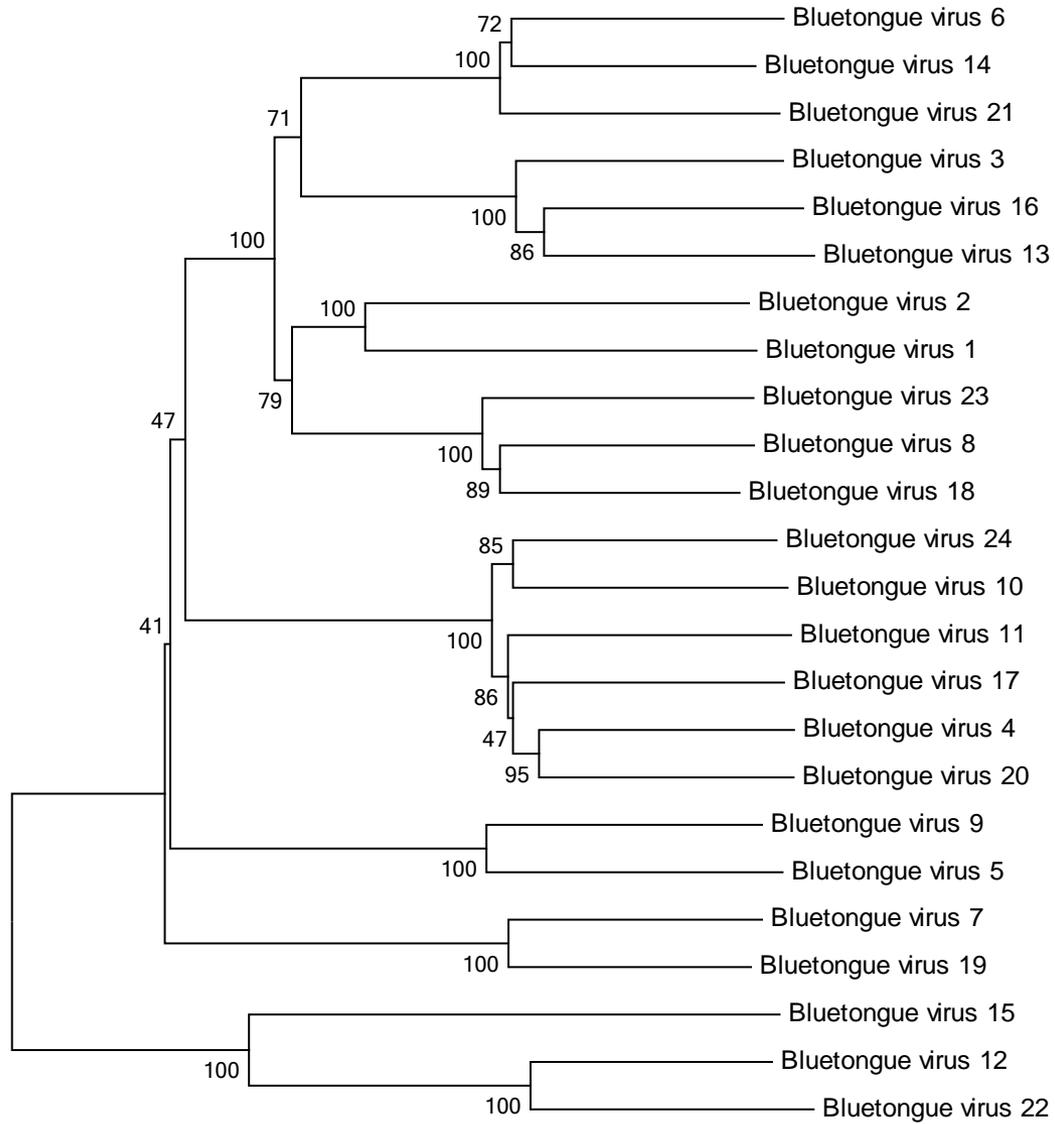
T=2
INNER CAPSID

BTV S2片段

根据已提交Genebank中的S2片段的序列，发现不同血清型的S2片段序列长度有较大的差异，在2909bp-2953bp之间变化，其中BTV-1 2940 bp、BTV-2 2943 bp、BTV-8 2939 bp、BTV-16 2929 bp。

BTV VP2蛋白

- S2片段编码病毒的壳体蛋白VP2，约为110 ku，在不同血清型病毒VP2的差异较大，氨基酸残基数在961-955之间变化。



0.1

- 对UniProt已校对的VP2氨基酸序列比对，发现序列一致性为23.71%，序列相似性为54.94 %。
VP2 存在许多能够诱导产生中和抗体的抗原表位，由于BTV不同血清型的VP2序列保守性低，因此疫苗研制面临很大的困难。

BTV S9与NS4

- Polly Roy 等人在过去的三十年里，认为病毒的分节基因组是单顺反子，编码病毒的VP6结构蛋白，但是2011年Massimo Palmarini等报道S9片段还编码一个小的非结构蛋白NS4。
- 目前NS4 的进一步研究已经展开

运用生物信息学分析

2008年生物信息学分析，预测了新的蛋白

Research

Open Access

Bioinformatic analysis suggests that the Orbivirus VP6 cistron encodes an overlapping gene

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实验验证

2011年12月，有两篇文章同时证明了NS4的存在

OPEN  ACCESS Freely available online

PLOS PATHOGENS

Identification and Characterization of a Novel Non-Structural Protein of Bluetongue Virus

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实验验证

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Detection of a Fourth Orbivirus Non-Structural Protein

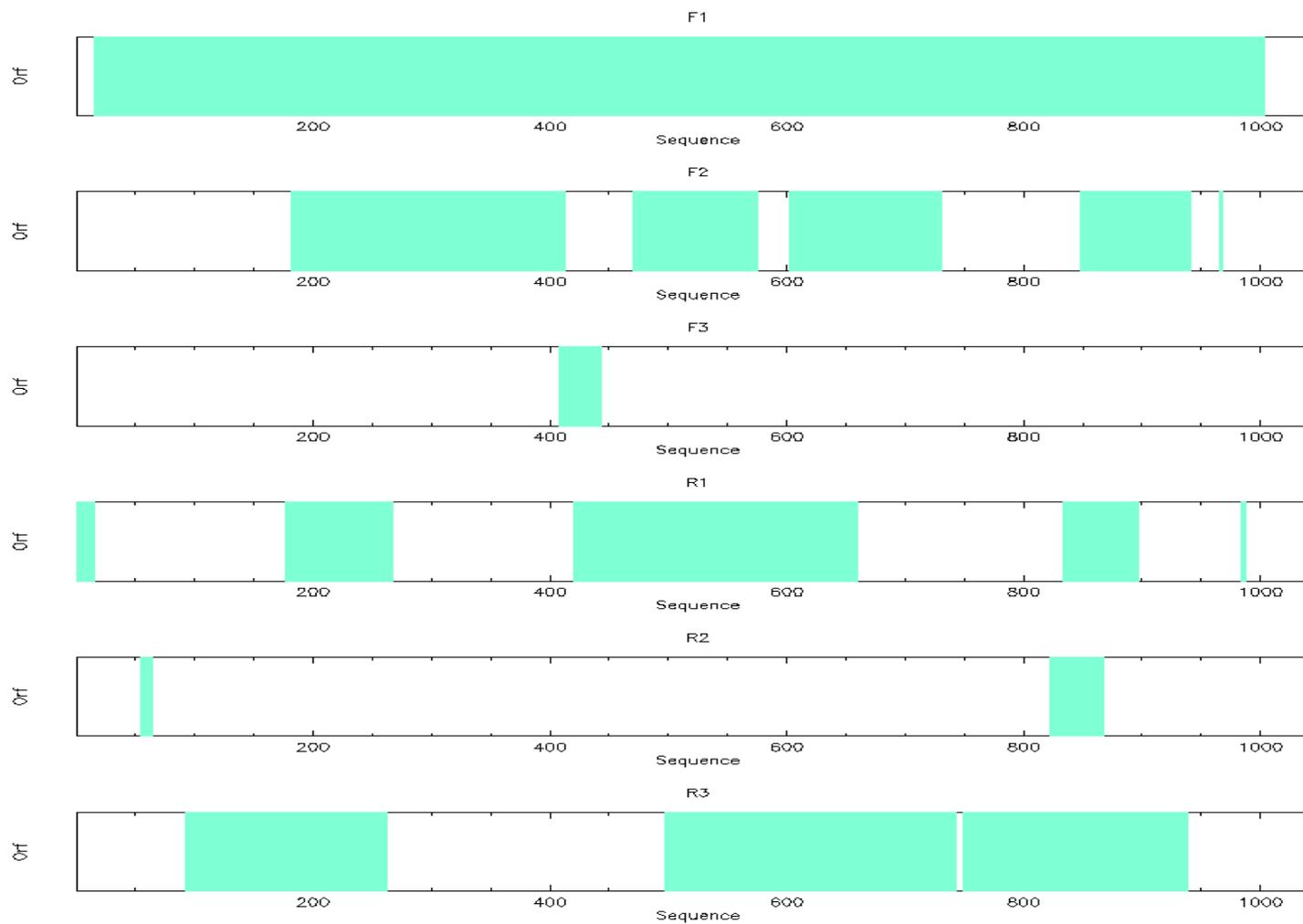
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Abstract

The genus *Orbivirus* includes both insect and tick-borne viruses. The orbivirus genome, composed of 10 segments of dsRNA, encodes 7 structural proteins (VP1–VP7) and 3 non-structural proteins (NS1–NS3). An open reading frame (ORF) that spans almost the entire length of genome segment-9 (Seg-9) encodes VP6 (the viral helicase). However, bioinformatic analysis recently identified an overlapping ORF (ORFX) in Seg-9. We show that ORFX encodes a new non-structural protein, identified here as NS4. Western blotting and confocal fluorescence microscopy, using antibodies raised against recombinant NS4 from Bluetongue virus (BTV, which is insect-borne), or Great Island virus (GIV, which is tick-borne), demonstrate that these proteins are synthesised in BTV or GIV infected mammalian cells, respectively. BTV NS4 is also expressed in *Culicoides* insect cells. NS4 forms aggregates throughout the cytoplasm as well as in the nucleus, consistent with identification of nuclear localisation signals within the NS4 sequence. Bioinformatic analyses indicate that NS4 contains coiled-coils, is related to proteins that bind nucleic acids, or are associated with membranes and shows similarities to nucleolar protein UTP20 (a processome subunit). Recombinant NS4 of GIV protects dsRNA from degradation by endoribonucleases of the RNase III family, indicating that it interacts with dsRNA. However, BTV NS4, which is only half the putative size of the GIV NS4, did not protect dsRNA from RNase III cleavage. NS4 of both GIV and BTV protect DNA from degradation by DNase. NS4 was found to associate with lipid droplets in cells infected with BTV or GIV or transfected with a plasmid expressing NS4.

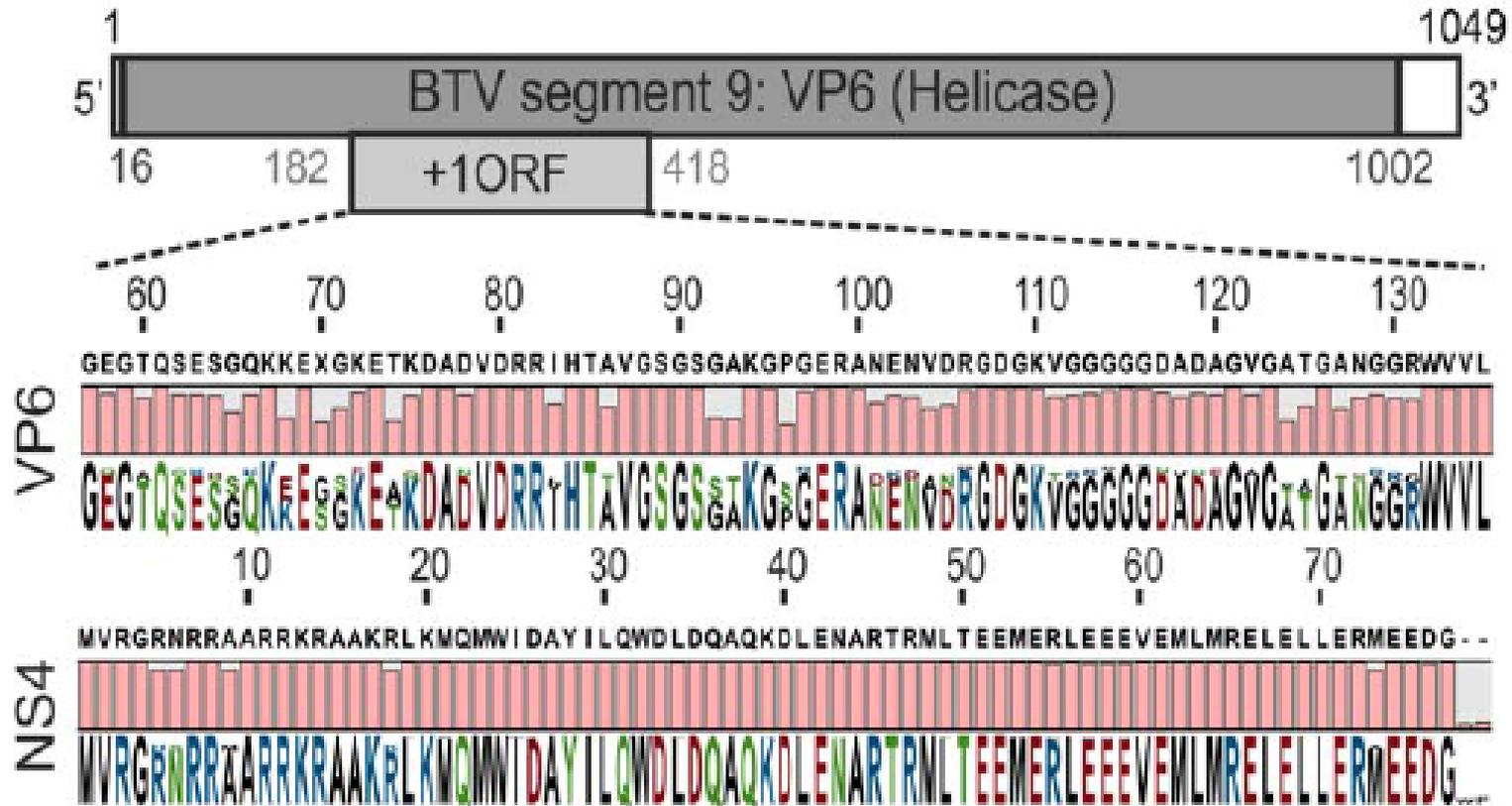
读码框分析



读码框分析

```
151 AAAGAGGAGAGCAAAGCAGAAGGATCAAAGGATGGTGAGGGGACGCAATC 200
F2 26 K R R A K Q K D Q R - H V R G R N R 42
-----|-----|-----|-----|-----|
201 GGAGAGCGGCCAGAAGGAAGAGGGCGGCAAAGAGACTAAAGATGCAGATG 250
F2 43 R A A R R K R A A K R L K M Q M 58
-----|-----|-----|-----|-----|
251 TGGATAGACGCATACATACTGCAGTGGGATCTGGATCAGGCACAAAAGGA 300
F2 59 W I D A Y I L Q W D L D Q A Q K D 75
-----|-----|-----|-----|-----|
301 TCTGGAGAACGCGCGAACGAGAATGCTAACAGAGGAGATGGAAAGGTTGG 350
F2 76 L E N A R T R M L T E E M E R L E 92
-----|-----|-----|-----|-----|
351 AGGAGGAGGTGGAGATGCTGATGCGGGAGTTGGAGCTACTGGAACGAATG 400
F2 93 E E V E M L M R E L E L L E R M 108
-----|-----|-----|-----|-----|
401 GAGGAAGATGGGTAGTTCTAACGGAAGAGATTGCTCGCGCGATTGAATCT 450
F2 109 E E D G * F * R K R L L A R L N L 10
```

重叠读码框



读码框分析

- >Bluetongue_8 [77 - 412] virus segment 9, complete sequence
- **NRGRFRLIWLIGWRVRAEKRIKQNLKRRRAKQKDQRMVRGRNRRA**
ARRKRAAKRLKMQMWIDAYILQWDLDDQAQKDLENARTRMLTEEM
ERLEEEVEMLMRELELLERMEEDG
- **MVRGRNRRAARRKR**AAKRLKMQMWIDAYILQWDLDDQAQKDLEN
ARTRMLTEEMERLEEEVEMLMRELELLERMEEDG
在猪瘟病毒中也出现有类似的核定位信号
- >Bluetongue_19 [1 - 1002] virus segment 9, complete sequence
- VKKSHMSAAILLAPGDVIKRSSEELKQRQIQINLVDWMESEGGKE
DKTEPKEESKAEGSKDGEGTQSESGQKEEGGKETKDADVDRRIH
TAVGSGSGTKGSGERANENANRGDGKVGGGGGDADAGVGATG
TNGGRWVVLTEEIARAIESKYGTKIDVYRDDVPAQIIEVERSLQKEL
GISREGVAEQTERLRDLRRKEKNGTHAKAVERGGRKQRKKAHGD
AQREGVEEEKTSEEPARIGITIEGVMSQKKLLSMIGGVERKMAPIG
ARESAVMLVSNNSIKDVVRATAYFTAPTGDPHWKEVAREASKKKN
LAYTSTGGDVKTEFLHLIDHL

启示与未来的问题

1. **NS4**的发现生物信息学对实验的指导作用，即使一个研究了**40**年的病毒，仍然可以发现新的东西。
2. **Polly Roy**等人研发的**VP6**缺失疫苗中只阐述了**VP6**的作用，现在新发现编码**NS4**蛋白编码基因也同时被缺失。

NS4与**VP6**到底是谁在起作用？

3. 在感染细胞中**NS4**含量很少是是有启动子封堵现象引起？