9eme Journée National Avicole

Le 8 et 9 Novembre, Hammamet - Tunisie

Outils moléculaires pour le contrôle de la maladie de Gumboro

Dr. Hicham Fathi

Directeur Régional Technique & Marketing, Aviculture Europe, MEA & CIS

HIPRA

La Referencia en Prevención para Salud Animal

Diagnostic moléculaire: IBDV

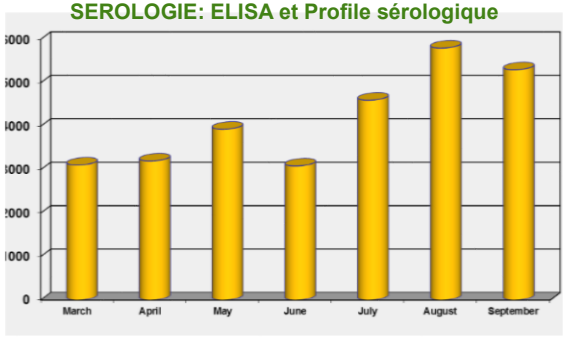
- Confirmer la présence virus IBDV après un passage ou une suspicion (POS/NEG): **Detection**
- Classification selon le degré de virulence: **Typage**
  - Classique non virulent (cvIBDV)
  - Très virulent (vvIBDV)
- **Séquençage** pour confirmer la classification et s'informer sur le niveau de parenté entre # virus (classique/vv/variant). **Phylogénie**
- Séquençage de la souche détecté et comparaison avec «Gen Bank» souche référence**: % d'homologie VP2 (probabilité).**

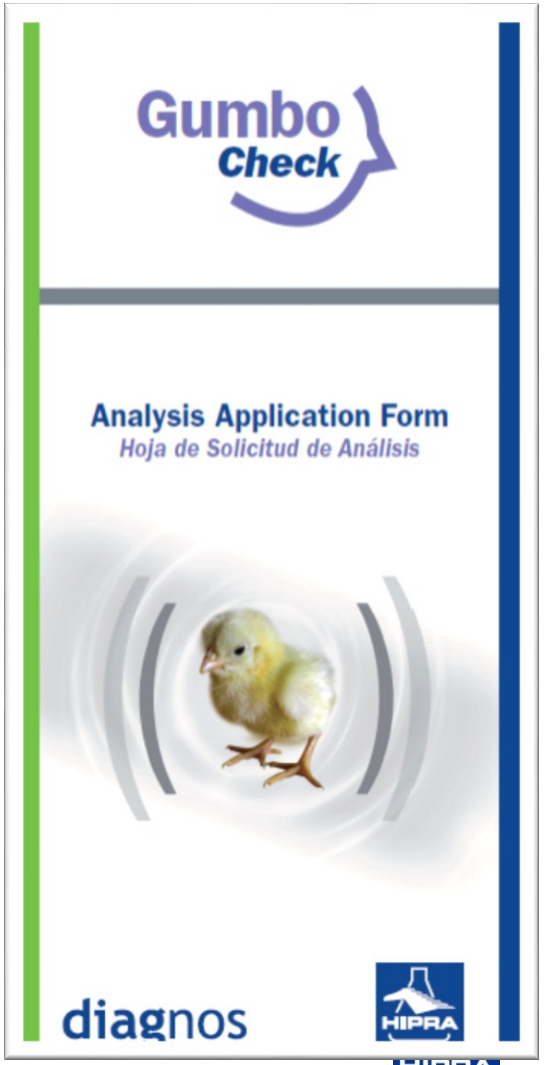
PROGRAMME HIPRAGUMBORO®

Support technique et DIAGNOS: Aaccompagnement de notre gamme Gumboro

LYMFOS TIMER
VACCINATION DAY CALCULATOR, by HIPRA

SEROLOGIE: ELISA et Profile sérologique





Gumbo Check: service de diagnostic moléculaire

Gumbo Check

Analysis Application Form
Hoja de Solicitud de Análisis

diagnos
your diagnostic service

HIPRA

sender details / *datos remitente*

Veterinarian-Technician responsible:
Veterinario-Técnico responsable: _____

Company:
Empresa: _____

Contact e-mail:
E-mail de contacto: _____

sample details / *datos muestra*

Farm Name:
Nombre de la granja: _____

Shed identification:
Identificación de la nave: _____

Town/City: _____ **Postcode:**
Población: _____ *Código Postal:* _____

Province: _____ **Country:**
Provincia: _____ *País:* _____

Date of sampling: / / **Age of birds (in days):**
Fecha toma de muestra: _____ *Edad de las aves (en días):* _____

Type of bird: *Tipo de ave:*
- ☐ Chicken *Pollos*
- ☐ Broiler Breeder *Reprod. Pesada*
- ☐ Layers *Ponedoras*
- ☐ Layer Breeder *Reprod. Ligera*

Sample Type: *Tipo de muestra:*
- ☐ Bursa imprint *Impresión de Bolsa*
- ☐ Other (please specify): *Otra (por favor especifique):* _____

ananlysis purpose / *motivo del análisis*

- ☐ Clinical diagnosis *Diagnóstico clínico*
- ☐ Vaccination control *Control vacunación*

clinical course / *proceso clínico*

Age of appearance (in days): *Edad de aparición (en días):* _____ **Mortality:** *Mortalidad:* ____ %

Morbidity: *Morbilidad:*
☐ < 10% ☐ 10%-30% ☐ > 30%

Symptoms: *Síntomas:*
☐ Depression *Depresión* ☐ Ruffled feathers *Plumas erizadas* ☐ Diarrhea *Diarrea*

Necropsy: *Necropsia:*
- ☐ Muscle petechia *Petequia muscular*
- ☐ Kidney lesion *Lesión renal*
- ☐ Edematic bursa *Bolsa edematosa*
- ☐ Hemorragic bursa *Bolsa hemorrágica*
- ☐ Atrophic bursa *Bolsa atrófica*

vaccination programme / *programa vacunal**
(Detail at least Gumboro vaccination / Detalle al menos la vacunació de Gumboro)

Age in days *Edad en días*	Vaccine (commercial product) *Vacuna (nombre comercial)*	*Administration Route *Vía de Administración*
		☐ ON ☐ OS ☐ OR

*ON Endonasal Endo-nasal OS Ocular Spray Spray OR Oral Oral

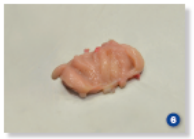
sampling protocol/ *protocolo de toma de muestras*

PLEASE READ THE PROTOCOL CAREFULLY BEFORE STARTING SAMPLE COLLECTION./*ANTES DE PROCEDER A LA RECOGIDA DE MUESTRAS LEA DETALLADAMENTE EL PROTOCOLO.*

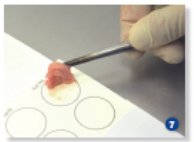
1. In case of an IBD outbreak take samples of birds with clinical symptoms. / *En caso de brote de IBD, recoja muestras de animales con sintomatología.*
2. Use one FTA® card properly identified with the farm name and shed ID for each shed sampled. / *Use una tarjeta FTA® debidamente identificada con el nombre de la granja y el código de la nave por nave muestreada.*
3. FTA® cards have four areas where the samples are applied (2 birds per area). Try not to rub the application surface. / *La tarjeta FTA® presenta cuatro áreas en las cuales aplicaremos las muestras, 2 aves por área. Evite dañar la superficie de la tarjeta FTA®.*

4. The preparation and handling of the samples should be performed in a clean area to avoid excessive contamination. Use of disposable gloves is required at all times when handling the samples. In any event, apply the basic principles for reducing contamination. / *La preparación y manejo de las muestras se debe realizar en una zona limpia. Utilice instrumental higienizado. Es necesario el uso de guantes desechables para evitar contaminaciones.*
5. Dissect the bursa of Fabricius. / *Diseccione la bolsa de Fabricio.*

6. Open the bursa by the middle to expose the inner mucosae. / *Abra la bolsa por el medio para exponer la mucosa interna.*



7. Smear the inner surface of the bursa against the middle of one of the circular areas. / *Frote la superficie interna de la bolsa contra el centro de una de las áreas circulares.*



8. Repeat the procedure in all areas to complete the FTA® card. 2 animals per area. / *Repita el mismo procedimiento en todas las áreas para completar la tarjeta FTA®. 2 animales por círculo.*

9. Allow the FTA® card to dry at room temperature in the position shown in the picture. / *Deje secar la tarjeta FTA® a temperatura ambiente en la posición que se muestra en la imagen.*

10. Once dried, place the FTA® card inside its plastic bag (along with the desiccant) and place it in the envelope provided, stored away from light. / *Una vez seca, introduzca la tarjeta FTA® en su bolsa de plástico (junto al secante) y póngala dentro del sobre facilitado, manteniéndolo al abrigo de la luz.*

Gumbo Check

HIPRA

Expérience Hipra en diagnostic moléculaire: IBDV

Diagnostique moléculaires IBDV depuis 2002

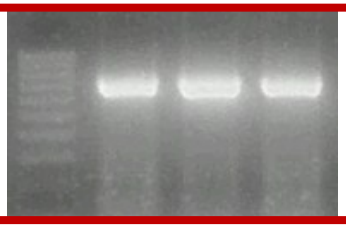
Echantillon de 43 pays différents de 2015 à 2017 (Diagnos)

Expérience Hipra en diagnostic moléculaire: IBDV

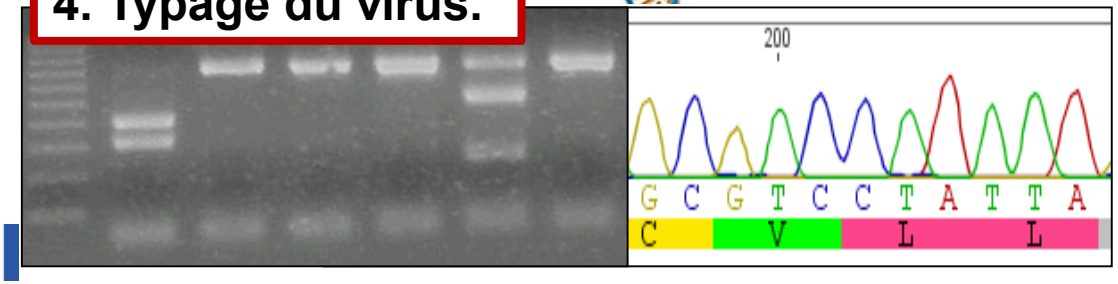
1. Prise d'échantillons sur carte FTA.

2. Extraction du matériel génétique .

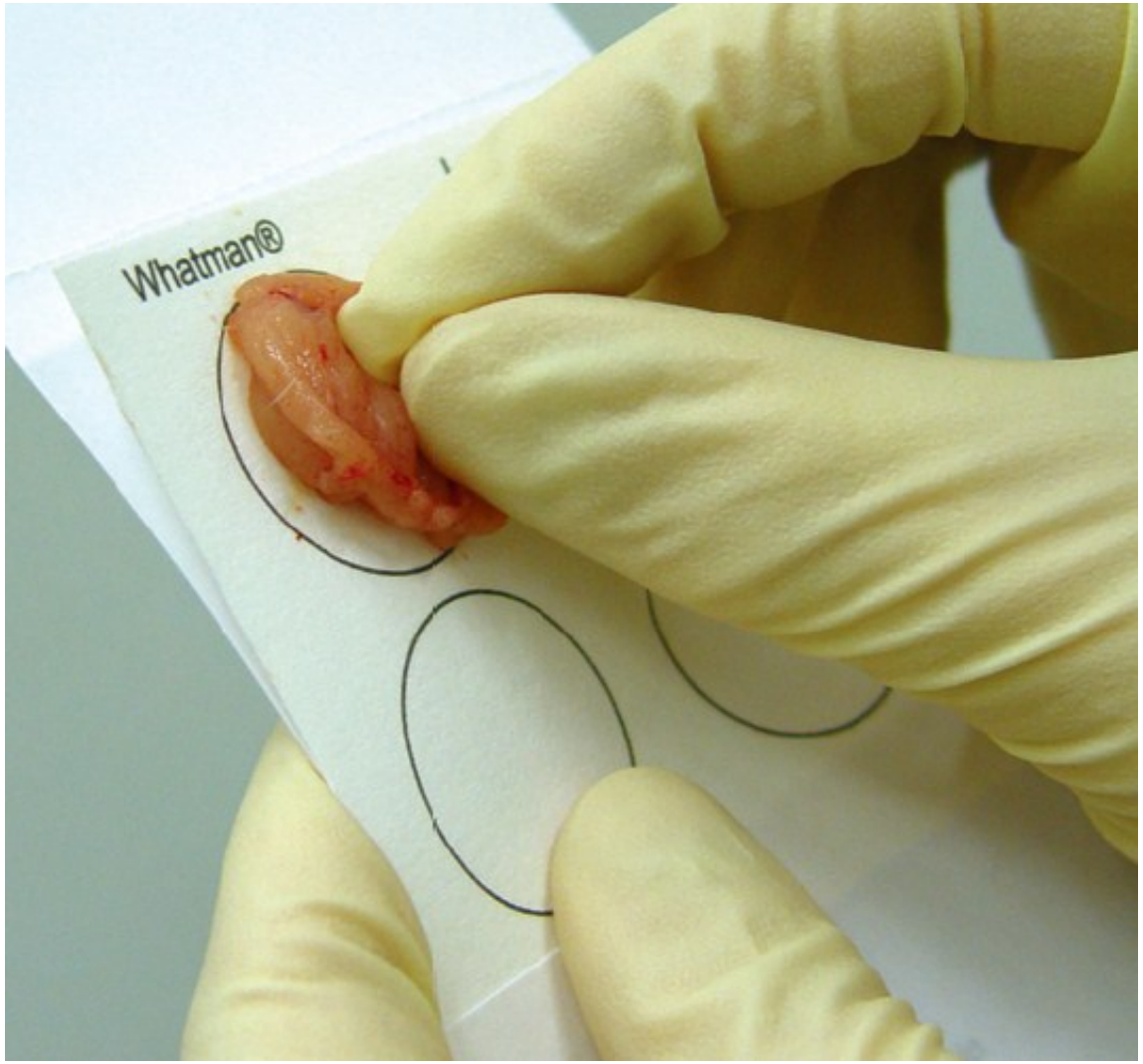
3. Amplification du matériel génétique IBDV . RT-PCR.



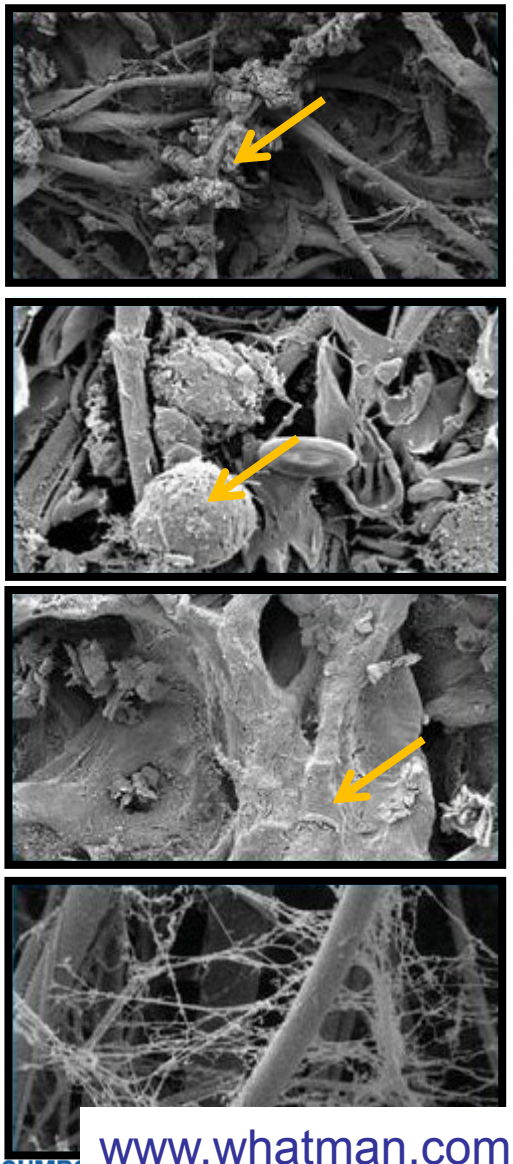
4. Typage du virus.



Echantillonnage sur carte FTA



Echantillonnage sur carte FTA



www.whatman.com

Composé chimique

Destruction capside virale & paroi bactérienne

Capture Matériel génétique

Pathogènes Mort
Accepté officiellement comme substance non infectieuse

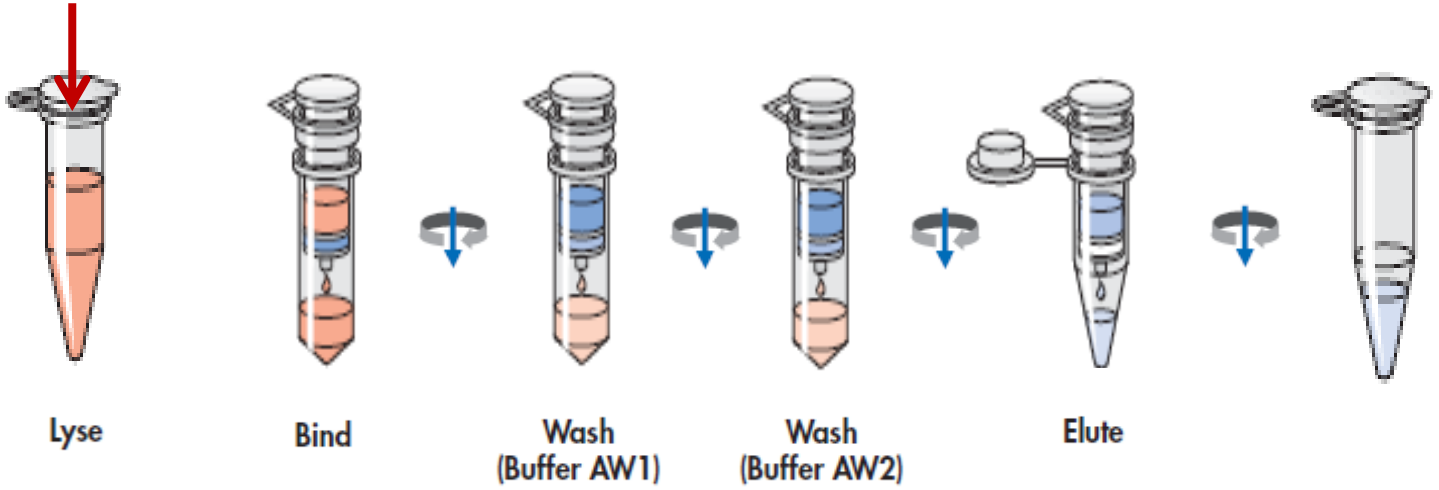
Matériel génétique **stable en Tº ambiante**

Envoie international facile

Extraction et purification du matériel génétique

- Matériel génétique ds l'échantillon (FTA): Broyage + PBS
- Purifier: élimination protéines, Protéase K
- Attachement de l'ARN: Poly A
- Lavage: élimination débris des cellules
- Elution: récupération de l'ARN

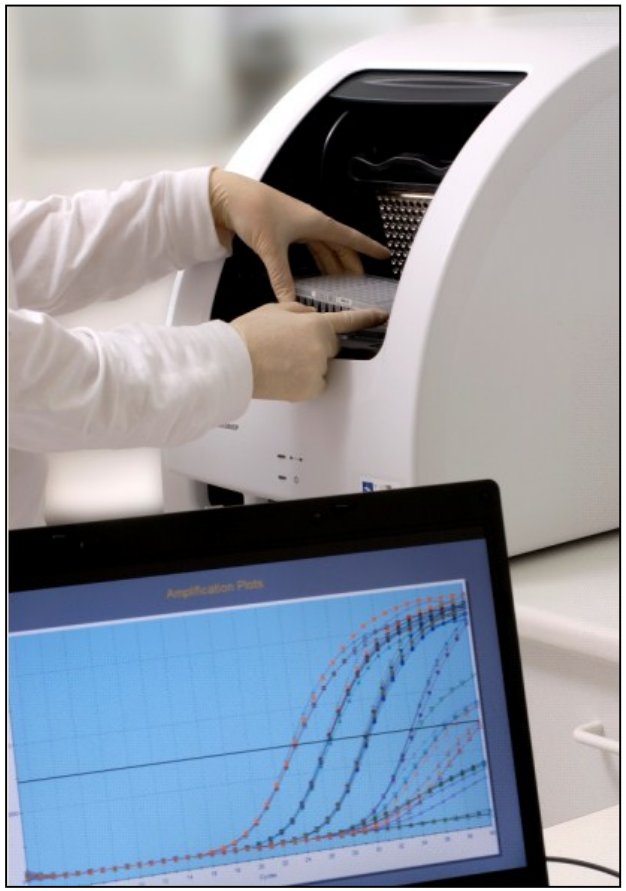
Echantillon + Composés chimique



RT-PCR. Amplification du matériel génétique

Reverse-Transcription Polimerase Chain Reaction (RT-PCR):

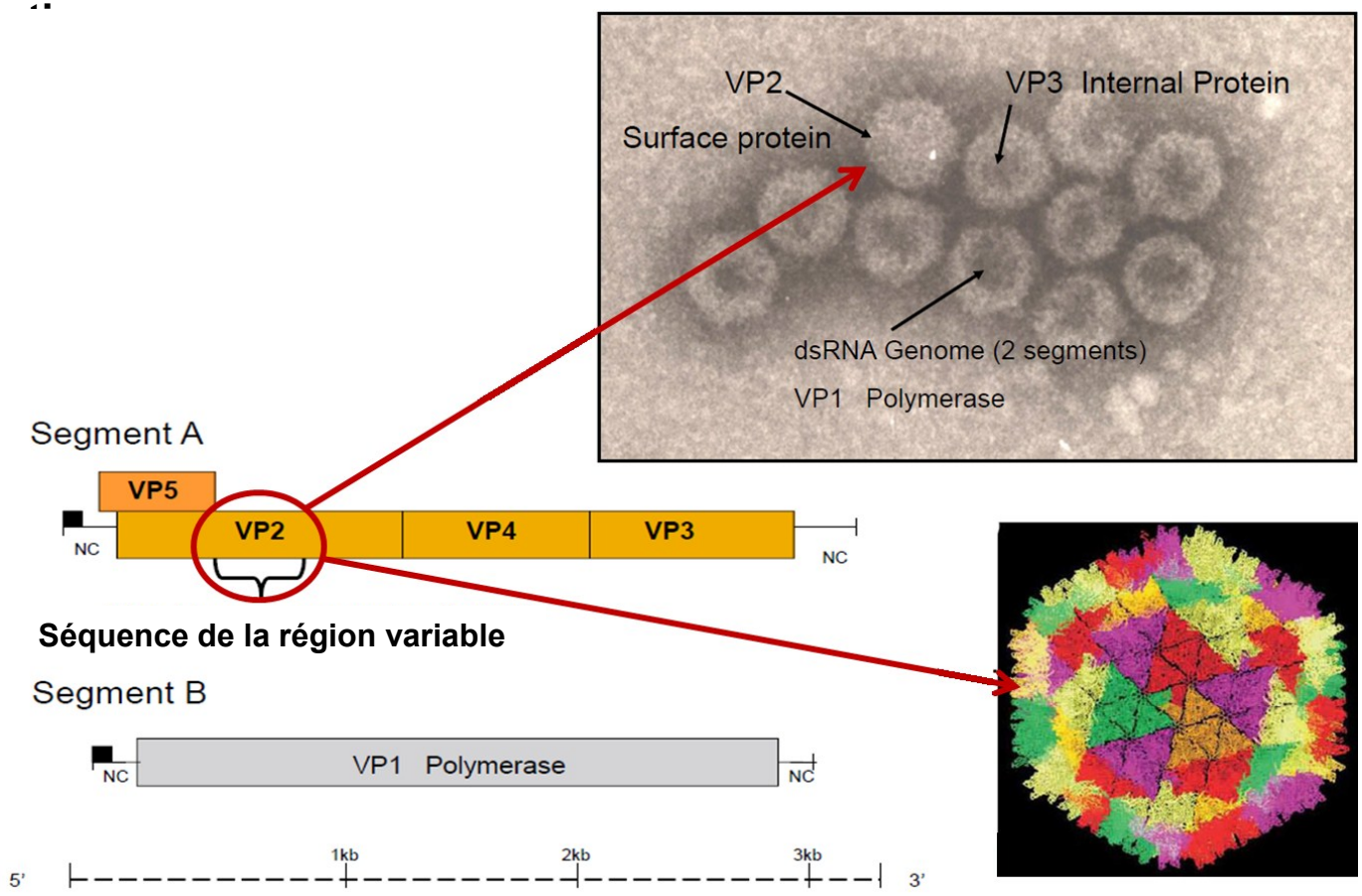
- Détection du génome IBDV
- Technique d'amplification très puissante
- ↑ Rapide et ↑ ↑ Sensitive
- Technique quantitative



RT-PCR. Amplification du matériel génétique

Les techniques moléculaires focalisent sur la détection d'une séquence de la portion hypervariable du gène VP2, qu'est composé de 345 nucléotides

- **Unique**
- **Conservée**
- **Inform**



VP2: La protéine la plus immunogène. Surface du virus

RT-PCR. Amplification du matériel génétique

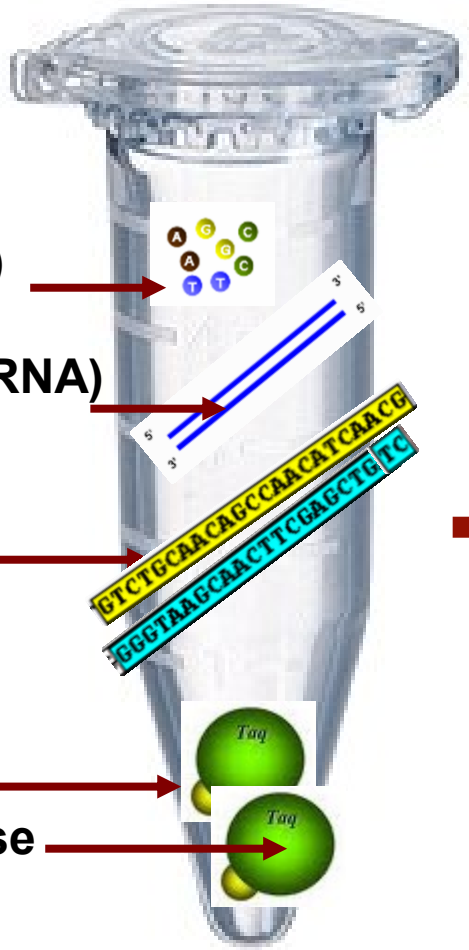
Gène VP2: 1368 bp
Amplification partielle: 686 bp (436-1122)

>Infectious bursal disease virus. Capsid protein VP2 **(VP2) gene**
(1368pb)

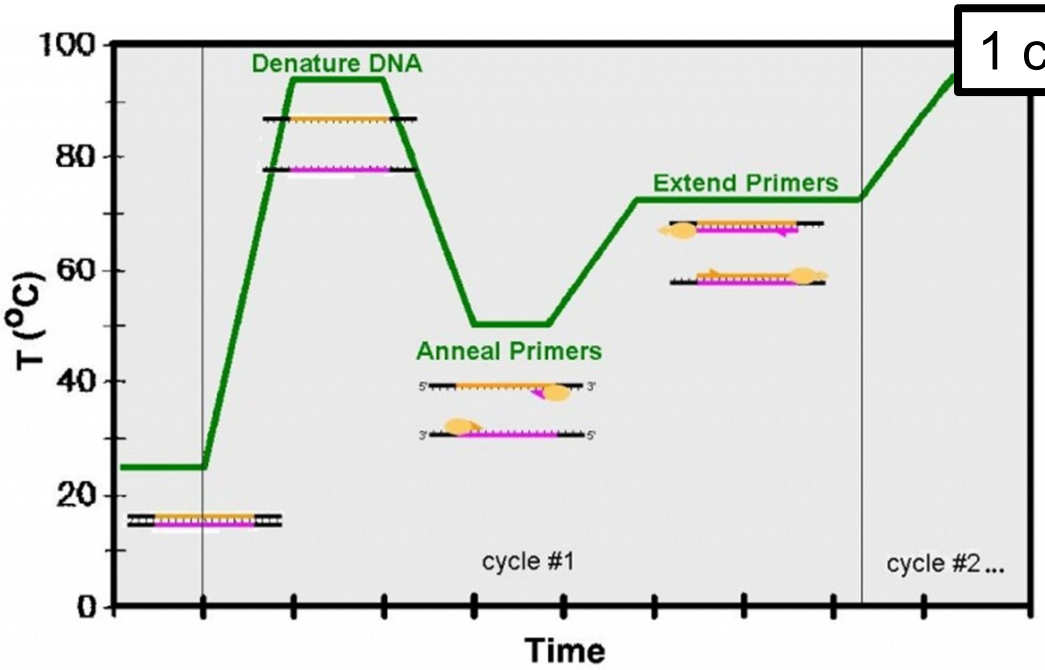
ATGGCGAACCTGCAAGATCAAACCCAACAGATTGTTCCGTTCA
GACCGGCGTCCATTCCGGACGACACCCTAGAGAAGCACACTCT
GACTGTGGGGGACACAGGGTCAGGGCTAATTGTCTTTTTCCCT
CACTACACACTGCAGAGCAATGGGAACTACAAGTTCGATCAGA
CCAGCTACAACTACTGCAGGCTAGTGAGTCGGAGTCTCACAGT
TTATGCACTAAATGGCACCATAAACGCCGTGACCTTCCGAGGAAGCCTGAGTGAACTGACAGATGTTAGC
TACAATGGGTTGA**GTCTGCAACAGCCAACATCAACG**CAAAATCAGGAACGTCCTAGTGGGGGAAGGGG
TGACCGTCCTCAGCTTACCCACATCATATGATCTTGGGTATGTGAGACTCGGTGACCCCATTCCCGCTAT
AGGGCTCGACCCAAAAATGGTAGCAACATGTGACAGCAGTGACAGGCCCAGAGTCTACACCATAACTGCA
GCCGATGATTACCAATTCTCATCACAGTACCAAGCAGGTGGGGTAACAATCACACTGTTCTCAGCTAATA
TCGATGCCATCACAAGCCTCAGCATCGGGGGAGAACTCGTGTTTCAAACAAGCGTCCAAGGCCTCATACT
GGGTGCTACCATCTACCTTATAGGCTTTGATGGGACTGCGGTAATCACCCGAGCTGTGCCCGCAGACAAT
GGGCTAACGGCCGGCACTGACAACCTTATGCCACTCAACATTGTGATTCCAACCAGCGAGATAACCCAGC
CAGTCACATCCATCAAACTGGAGATAGTGACCTCCAAAAGTGGTGGCCAGGCGGGGGATCAGATGTCATG
GTCAGCAAGTGGGAGCCTAGCAGTGACGATCCACGGTGGCAACTATCCAGCGGCCCTCCGTCCCGTCACA
CTAGTAGCCTACGAAAGAGTGGCAACAGGGTCTGTCGTTACGGTCGCCG**GGGTAAGCAACTTCGAGCTGA**
TCCCAAATCCTGAACTAGCAAAGAACCTGGTCACAGAATACGGCCGATTTGACCCAGGGGCCATGAACTA
CACAAAATTGATACTGAGTGAGAGGGACCGTCTTGGCATCAAGACCGTATGGCCAACAAGGGAGTACACT
GACTTTCGCGAGTACTTCATGGAGGTGGCCGACCTCAACTCTCCCCTGAAGATTGCAGGAGCATTTGGCT
TCAAAGACATAATCCGGGCCATAAGGAGGTAGTAGAAT

Design des Amorces
Unique pour IBDV
Conservé ds tte les souches IBDV
Fragment hypervariable

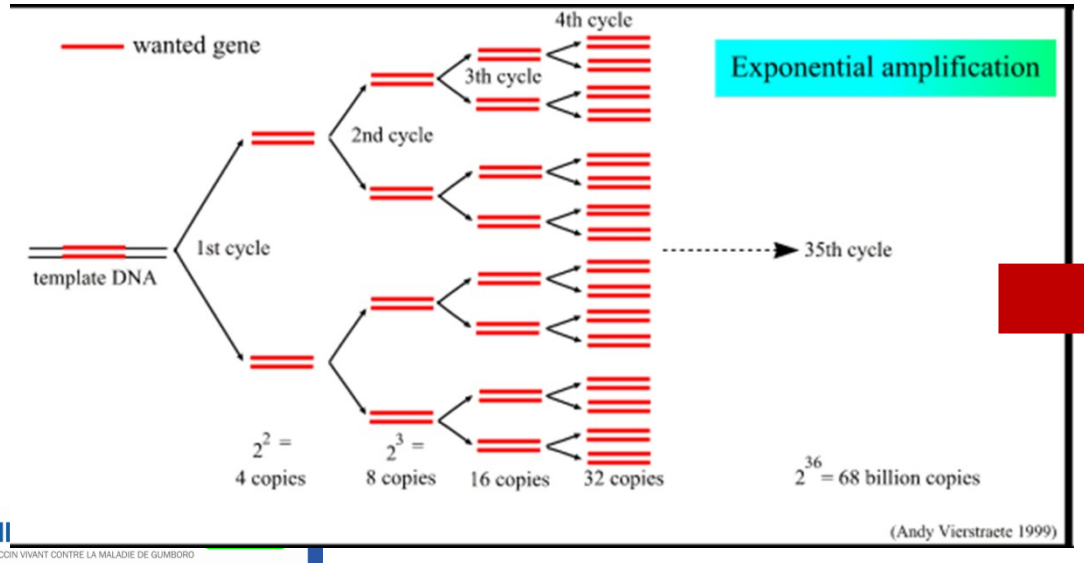
RT-PCR. Amplification du matériel génétique

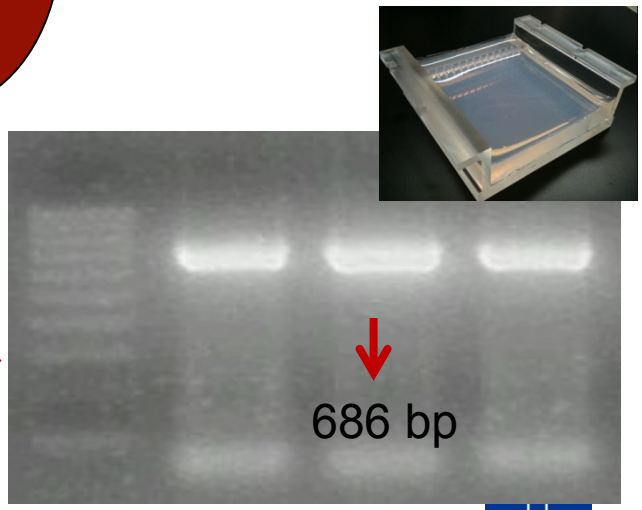


RT-PCR. Amplification du matériel génétique



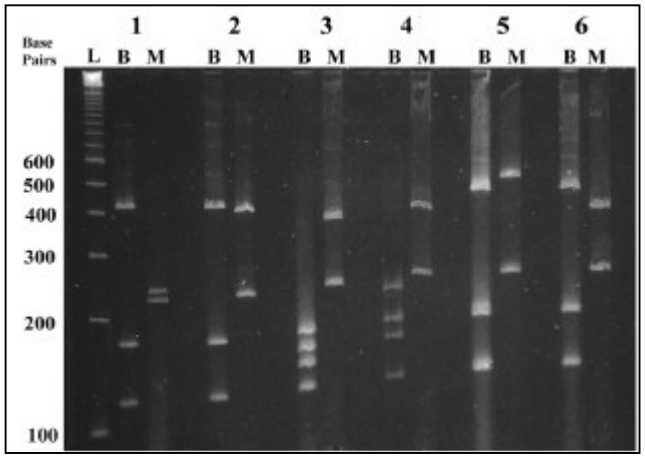
X 35-40 cycles

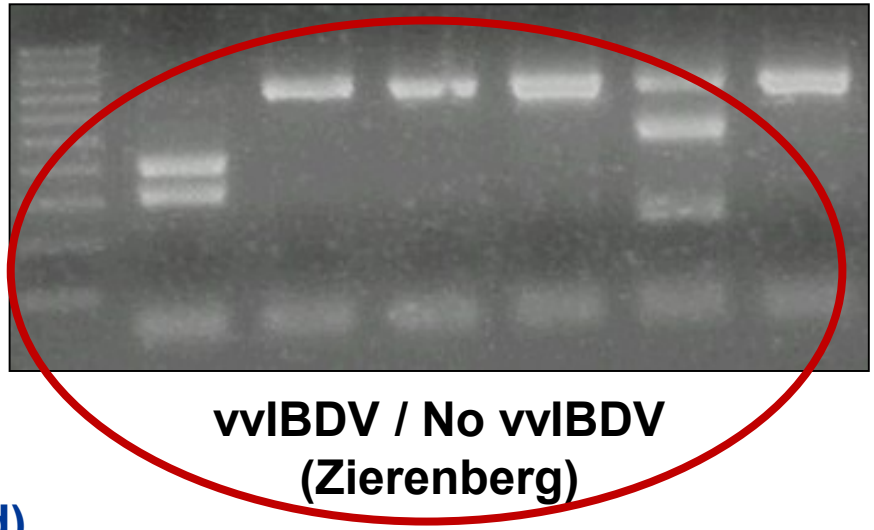




Typage IBDV

- Restriction enzymatique





vvIBDV / No vvIBDV (Zierenberg)

6 groupes moléculaire (Jackwood)

Avian Dis. 2001 Apr-Jun;45(2):330-9.

Amino acid comparison of infectious bursal disease viruses placed in the same or different molecular groups by RT/PCR-RFLP.

Jackwood DJ[1], Sommer SE, Knoblich HV.

Avian Pathol. 2001 Feb;30(1):55-62. doi: 10.1080/03079450020023203.

Rapid identification of "very virulent" strains of infectious bursal disease virus by reverse transcription-polymerase chain reaction combined with restriction enzyme analysis.

Zierenberg K[1], Raue R, Muller H.

Typage IBDV

Restriction Enzymatique

PCR PRODUCT: 686 nt VP2 gene

Sac I (NoVV): GAGCT'C (2 fragments: 303nt and 383nt)

BspMI (VV): 'GCAGGT (2 fragments: 388nt and 298nt)

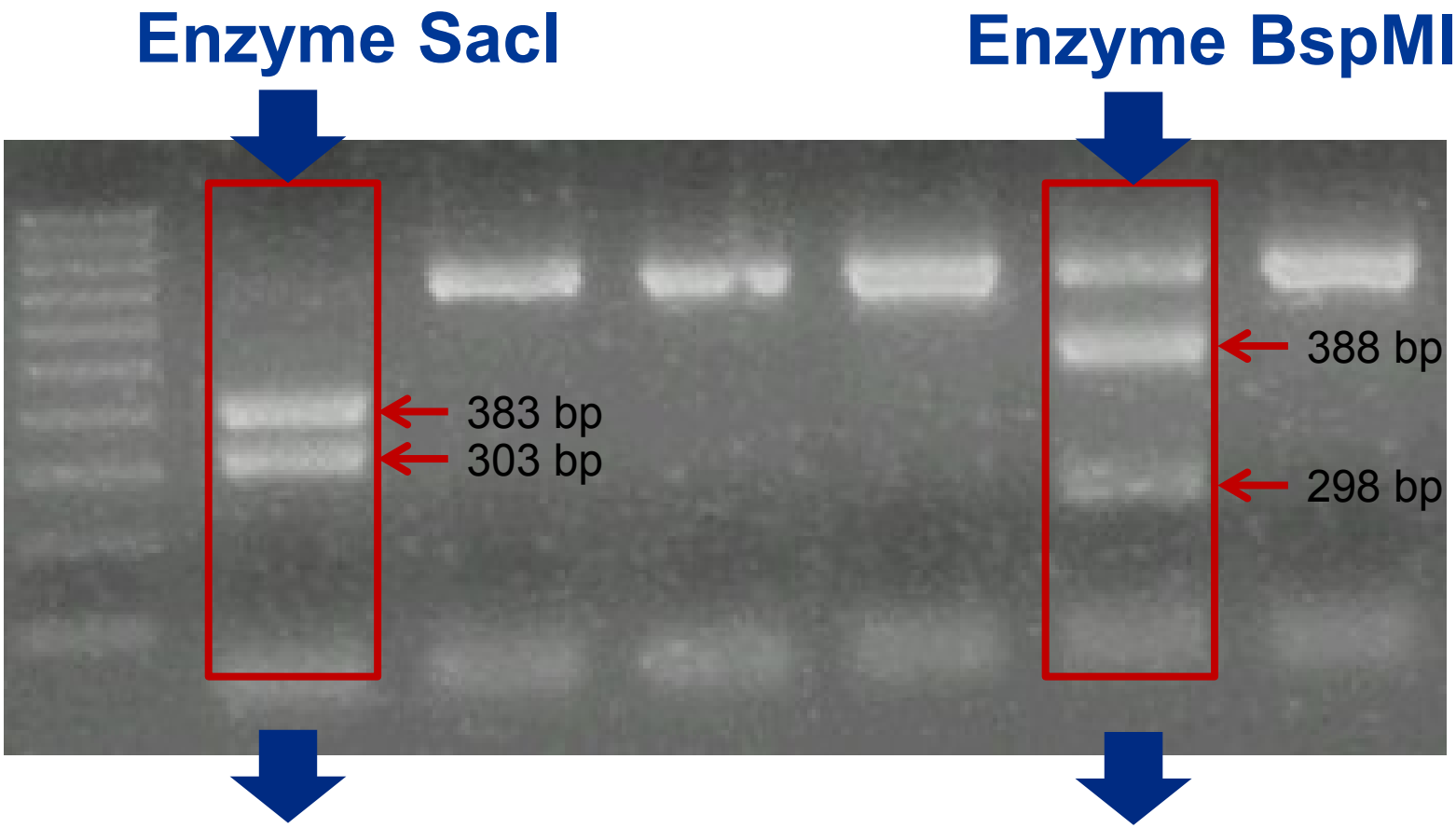
>**NoVV IBDV.** Partial VP2

GTCTGCAACAGCCAACATCAACGACAAAATTGGGAACGTCCTAGTAGGGGAAGGGGTAACCGTCCTCAGC
TTACCCACATCATATGATCTTGGGTATGTGAGGCTTGGTGACCCCATACCCGCTATAGGGCTTGACCCAA
AAATGGTAGCAACATGTGACAGCAGTGACAGGCCCAGAGTCTACACCATAACTGCAGCCGATAATTACCA
ATTCTCATCACAGTACCAA**A**CAGGTGGGGTAACAATCACACTGTTCTCAGCCAACATTGATGCCATCACA
AGTCTCAGCGTTGGGGGA**GAGCTC**GTGTTCAAAACAAGCGTCCAAAGCCTTGTACTGGGCGCCACCATCT
ACCTTATAGGCTTTGATGGGACTGCGGTAATCACCAGAGCTGTGGCCGCAAACAATGGGCTGACGGCCGG
CATCGACAATCTTATGCCATTCAATCTTGTGATTCCAACCAATGAGATAACCCAGCCAATCACATCCATC
AAACTGGAGATAGTGACCTCCAAAAGTGATGGTCAGGCAGGGGAACAGATGTCATGGTCGGCAAGTGGGA
GCCTAGCAGTGACGATCCATGGTGGCAACTATCCAGGAGCCCTCCGTCCCGTCACACTAGTGGCCTACGA
AAGAGTGGCAACAGGATCTGTCGTTACGGTCGCTG**GGGTGAGCAACTTCGAGCTGA**

>**VV IBDV.** Partial VP2

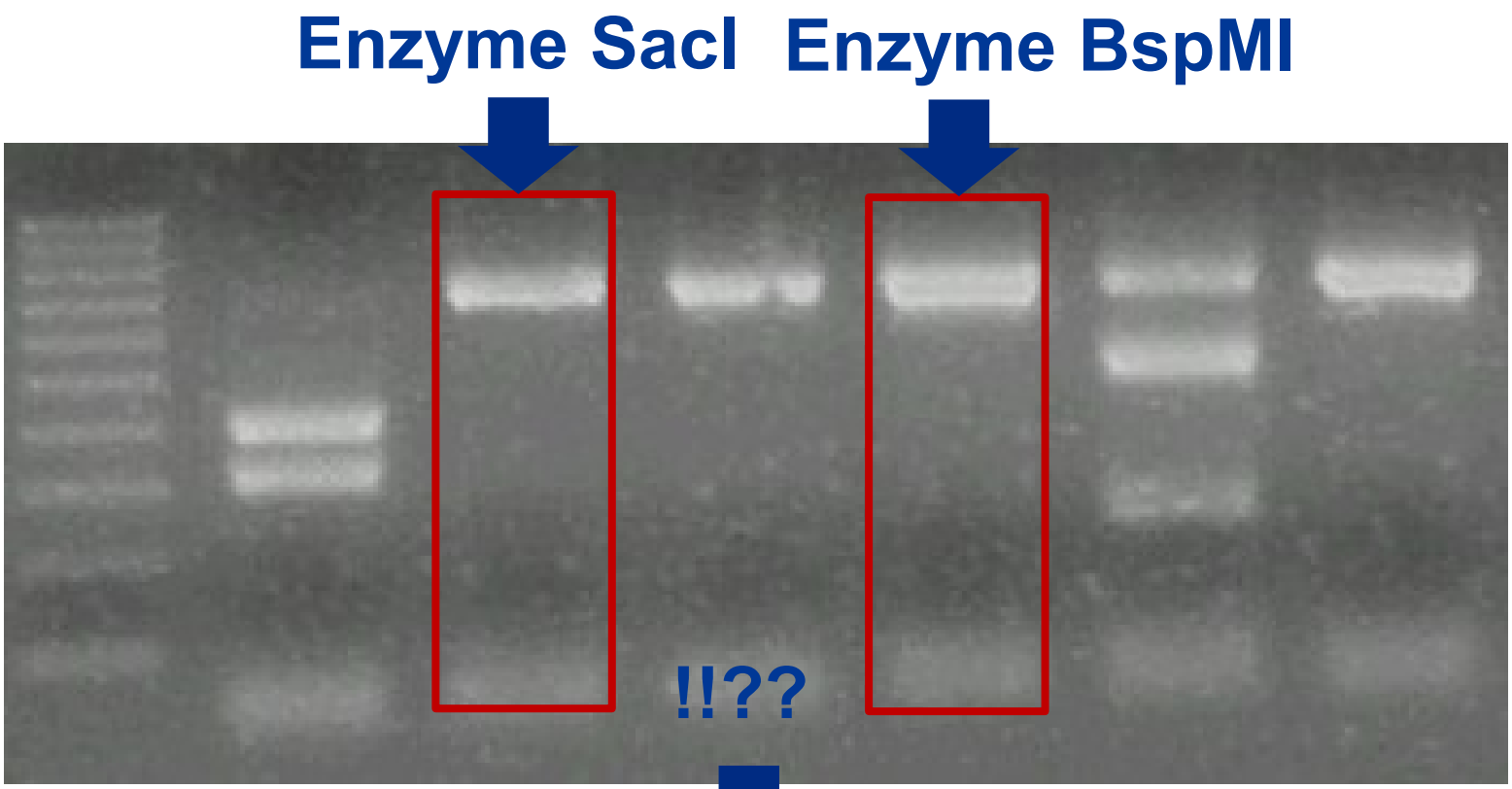
GTCTGCAACAGCCAACATCAACGACAAAATCGGGAACGTCCTAGTAGGGGAAGGGGTAACCGTCCTCAGC
TTACCCACATCATATGATCTTGGGTATGTGAGACTCGGTGACCCCATTCCCGCTATAGGGCTCGACCCAA
AAATGGTAGCAACATGTGACAGCAGTGACAGGCCCAGAGTCTACACCATAACTGCAGCCGACGATTACCA
ATTCTCATCACAGTACCAA**GCAGGT**GGGGTAACAATCACACTGTTCTCAGCTAATATCGATGCCATCACA
AGCCTCAGCATCGGGGGAGA**A**CTCGTGTTTCAAACAAGCGTCCAAGGCCTTATACTGGGTGCTACCATCT
ACCTTATAGGCTTTGATGGGACTGCGGTAATCACCAGAGCTGTGGCCGCAGACAATGGGCTGACGGCCGG
CACTGACAACCTTATGCCATTCAATATTGTGATTCCAACCAGCGAGATAACCCAGCCAATCACATCCATC
AAACTGGAGATAGTAACCTCCAAAAGTGGTGGTCAGGCGGGGGATCAGATGTCATGGTCAGCAAGTGGGA
GCCTAGCAGTGACGATCCACGGTGGCAACTATCCAGGGGCCCTCCGTCCCGTCACACTAGTAGCCTACGA
AAGAGTGGCAACAGGATCTGTCGTTACGGTCGCC**GGGGTGAGCAACTTCGAGCTGA**

Typage IBDV


Enzyme SacI
Enzyme BspMI
383 bp
303 bp
388 bp
298 bp
No vvIBDV
vvIBDV

Typage IBDV

Mais parfois…



Typage IBDV

diagnos
your diagnostic service

HIPRA MALAYSIA SDN BHD

[redacted],
[redacted]DR
Malaysia

Dr/a.

Amer, 23 February 2.011

Report: **A00019537**
Species: Poultry

Farm: ~~HASS~~

Received samples

Amount	Sample	Category	Age	Reference
1	FTA			

Results:

MOLECULAR DIAG.

W/R: Without reference FS: Not tested due to lack of sample.

Infectious Bursal Disease

FTA - Poultry

W/R - **POS**

COMMENTS:

It was not possible to characterize the strain of IBDV detected in the sample with the performed methodology:
Restriction Fragment Length Polimosphysm (RFLP).

Attached, please find results from phylogenetic analysis of IBDV.

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your diagnostic service

Email: [redacted]

HIPRA POLSKA Sp.z.o.o.

[redacted]

Poland

[redacted]

Amer, 21 September 2.011

Report: **A00025403**
Species: Poultry

Farm: [redacted]

Received samples

Amount	Sample	Category	Age	Reference
1	FTA	Broiler	25 Days	

Results:

MOLECULAR DIAG.

W/R: Without reference FS: Not tested due to lack of sample.

Infectious Bursal Disease

FTA - Broiler - 25 Days
W/R - **POS**

COMMENTS:

It was not possible to characterize the strain of IBDV detected .

Attached, please find results from phylogenetic analysis of IBDV.

HIPRA

Typage IBDV

HIPRA diagnos your diagnostic service

Email: [redacted]

HIPRA SOUTHERN AFRICA [redacted]

Amer, 2 May 2.012

Report: **A00031066**

Species: Poultry

South Africa

Farm: [redacted]

Received samples

Amount	Sample	Category
1	FTA	Broiler
1	FTA	Broiler
1	FTA	Broiler
1	FTA	Broiler
1	FTA	Broiler
1	FTA	Broiler
1	FTA	Broiler
1	FTA	Broiler
1	FTA	Broiler
1	FTA	Broiler

Infectious Bursal Disease virus typing

FTA - Broiler - 34 Days (SITE 1)
W/R - **E-%Nontypeable**

FTA - Broiler - 34 Days (SITE 2)
W/R - **E-%Nontypeable**

FTA - Broiler - 34 Days (SITE 4 (A,B,C))
W/R - **E-%NO vvIBDV**

FTA - Broiler - 34 Days (SITE 6)
W/R - **E-%Nontypeable**

FTA - Broiler - 34 Days (SITE 8)
W/R - **E-%NO vvIBDV**

FTA - Broiler - 34 Days (SITE 9)
W/R - **E-%NOvvIBDV**
vvIBDV

FTA - Broiler - 34 Days (JR)
W/R - **E-%Nontypeable**

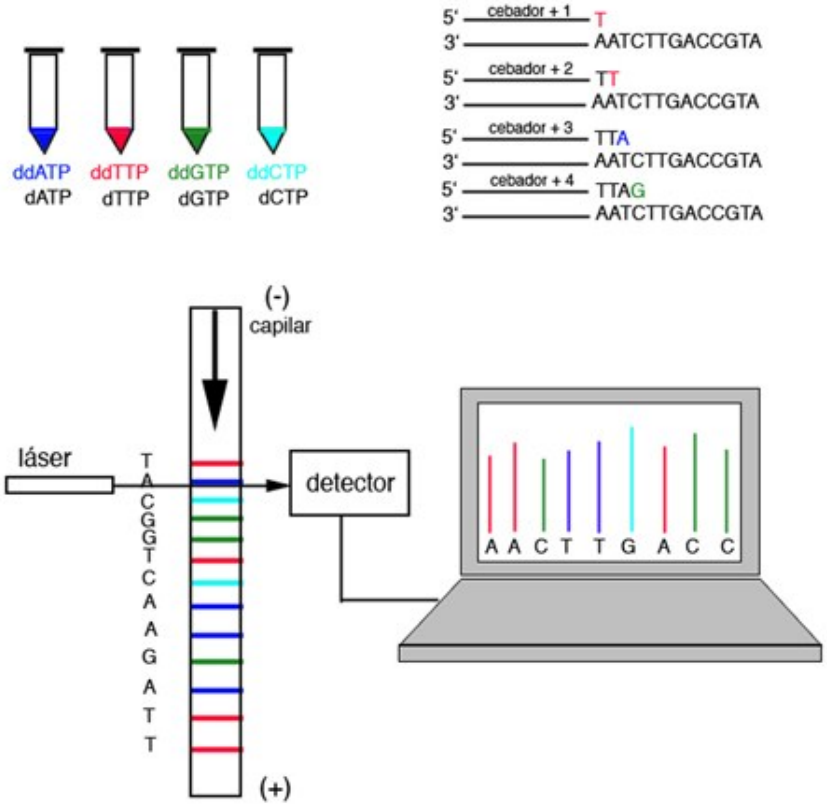
FTA - Broiler - 34 Days (SOLAR 3)
W/R - **E-%NO vvIBDV**

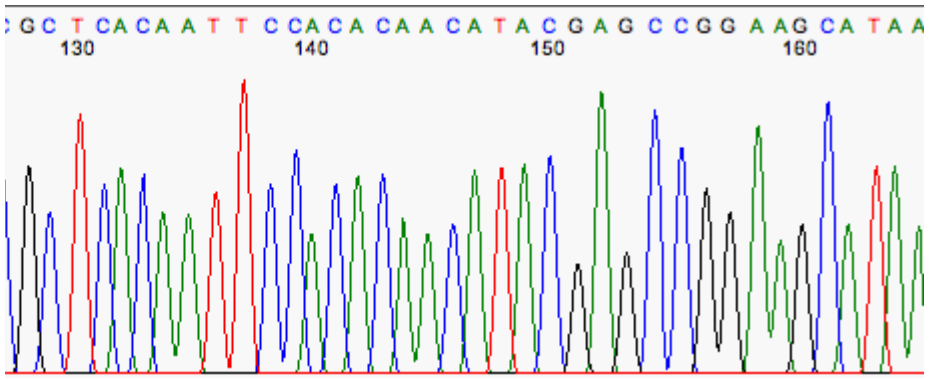
FTA - Broiler - 34 Days (MOUNTAIN VIELO 2)
W/R - **E-%Nontypeable**

34 Days	MOUNTAIN VIELO 2

Typage IBDV

Séquençage du Génome





Pour obtenir la séquence du nucléotide du fragment amplifier du virus

Typage IBDV

31066
South Africa

>31066-6-VP2-NoVVIBDV-SudAfrica-abril2012 VV vsNoVV
TGACCCCATTCCCGCTATAGGGCTCGACCCAAAAATGGTAGCAACATGTGA
CAGCAGTGACAGGCCCAGAGTCTACACCATAACCGCAGCTGATGATTACCA
ATTCTCATCACAGTACCAAGAAGGTCGGGTGACAATCACACTGTTCTCAGC
AAATATCGATGCCATCACAAGCCTCAGCATCGGGGGCAGAACTCGTGTTTCA
AACAAGTGTCCAAAGCCTTATACTTGGAGCTACCATCTACCTTATAGGCTTT
GATGGGACTGAGGTAATCACCAGAGCTGTAGCCGCAGACAATGGGCTAACG
GCCGGCACTGACAACCTTATGCCATTCAATATTGTGATTCCAACCAGCGAGA
TAACCCAGCCAATCACATCCATCAAACTGGAGATAGTAACCTCCAAAAGTG
GTGGTCAGGCGGGGGATCAGATGTCATGGTCAGCTAGTGGGAGCTTAGCAG
TGACGATCCACGGCGGCAACTAT

BLAST

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
JF965438.1	Infectious bursal disease virus isolate LD-847-04 VP2 protein precurs	817	817	100%	0.0	97%
AF527039.1	Infectious bursal disease virus strain UPM94/273 VP5 and polyproteir	817	817	100%	0.0	97%
AF248612.1	Infectious bursal disease virus /UPM94/273 seqment A VP2 protein ('	817	817	100%	0.0	97%
AF159217.1	Infectious bursal disease virus K280/89 seqment A VP2 qene, partial	817	817	100%	0.0	97%
AY628215.1	Infectious bursal disease virus isolate P8G VP2 (VP2) qene, partial cc	811	811	100%	0.0	97%

```
FEATURES             Location/Qualifiers
     source          1..642
                     /organism="Infectious bursal disease virus"
                     /mol_type="genomic RNA"
                     /strain="K280/89"
                     /db_xref="taxon:10995"
                     /chromosome="segment A"
                     /country="South Africa"
                     /note="very virulent; isolated from bursa in 1988"
```

Typage IBDV

Infectious Bursal Disease virus typing

FTA - Poultry (WINT2512 LOT 30)

W/R - **NO vvIBDV**

(By enzyme restriction analysis)

24782
Russie

>24782-17-F_Russia_VV/NoVV

CTTACCCACATCATATGATCTTGGGTATGTGAGACTCGGTGACCCCATTCCCGCTATAGGGCTCGACC**C**A
AAAATGGTAGCAACATGTGACAGCACTGACAGGCCCAGAGTCTACACCATAACTGCAGCCGATGATTACC
AATTCTCATCACAGTACCAAGCAGGTGGAGTAACAATCACACTGTTCTCAGCTAACATCGATGCCATCAC
AAGCCTCAGCATCGGGGGAGAACTCGTGTTTCAAACAAGCGTCCAAGGCCTTATACTGGGCGCTACCATC
TACCTTATAGGCTTTGATGGGACCGGGTAACCACCAGAGCTGTGGCCGCAAACAATGGGCTAACGACCG
GCACTGACAACCTTATGCCATTCAATATTGTGATTCCAACCAGCGAGATAACCCAGCCAATCACATCCAT
CAAACTGGAGATAGTGACCTCCA**N**AAGTGGTGGTCAGGCGGGGGATCAGATGTCATGGTCAGCAAGTGGG
AGCCTAGCAGTGACGATCCA**C**GGTGGCAACTATCCAGGGGCCCTCCGTCCCGTCACACTAGT**A**GCCTACG
AAAGAGTGGCAACAGGATCTGTCGTTACGGTC

Souche IBDV atténuée

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
DQ927040.1	Infectious bursal disease virus strain mb segment A polyprotein mRN/	1079	1079	100%	0.0	99%
AF457103.1	Infectious bursal disease virus strain ABIC/MB71 VP2 protein gene, p	1074	1074	100%	0.0	99%
DQ927042.1	Infectious bursal disease virus strain ks segment A polyprotein mRNA	1068	1068	100%	0.0	99%
DQ825652.1	Infectious bursal disease virus strain HZ polyprotein mRNA, partial cc	1062	1062	100%	0.0	99%
DQ450988.1	Infectious bursal disease virus polyprotein gene, partial cds	1062	1062	100%	0.0	99%

Typage IBDV

25403
Pologne

FTA - Brojlery - 25 Dni
B/O - **POS**

Komentarze:

It was not possible to characterize the strain of IBDV detected .

>25403_Polonia_VV_no digiere_setembre2011

CCAACATCAACGACAAAATCGGGAACGTCCTAGTAGGGGAAGGGGTAACCGTCCTCAGCTTACCCACATC
ATATGATCTTGGGTATGTGAGACTCGGTGACCCCATTCCCGCCATAGGGCTCGACCCAAAAATGGTAGCA
ACATGTGACAGCAGTGACAGGCCCAGAGTCTACACCATAACTGCAGCCGATGATTACCAATTCTCATCAC
AGTACCAAGCAGGAGGGGTAACAATCACACTGTTCTCAGCCAATATCGATGCCATCACAAGCCTCAGTAT
CGGGGGAGAACTCGTGTTTCAAACAAGTGTCCAAGGCCTTATACTGGGTGCTACCATCTACCTTATAGGC
TTTGATGGGACTACGGTAATCACCAGAGCTGTGGCCGCAGACAATGGGCTAACGGCCGGCACTGACAACC
TTATGCCATTCAACATTGTGATTCCAACCAGCGAGATAACCCAGCCAATCACATCCATCAAACTGGAGAT
AGTGACCTCCAAAAG

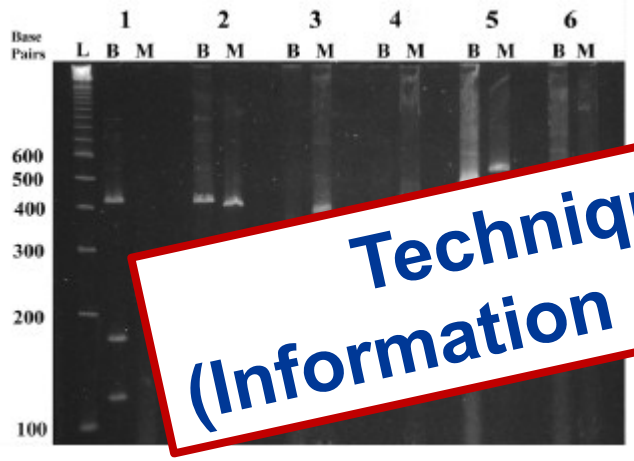
Souche vvIBDV

BLAST

Infectious bursal disease virus, segment A, isolate SH99	889	889	100%	0.0	98%
Infectious bursal disease virus segment A, complete genome, strain 89163	889	889	100%	0.0	98%
Infectious bursal disease virus strain 3529/92 polyprotein mRNA, complete cds	889	889	100%	0.0	98%
Infectious bursal disease virus isolate HeN-h VP2 mRNA, partial cds	889	889	100%	0.0	98%
Infectious bursal disease virus isolate P8G VP2 (VP2) gene, partial cds	889	889	100%	0.0	98%

Typage IBDV

Restriction enzymatique.



Technique ancienne (Information génétique limitée)

vvIBDV / No vvIBDV (Zierenberg)

6 groupes moléculaire (Jackwood)

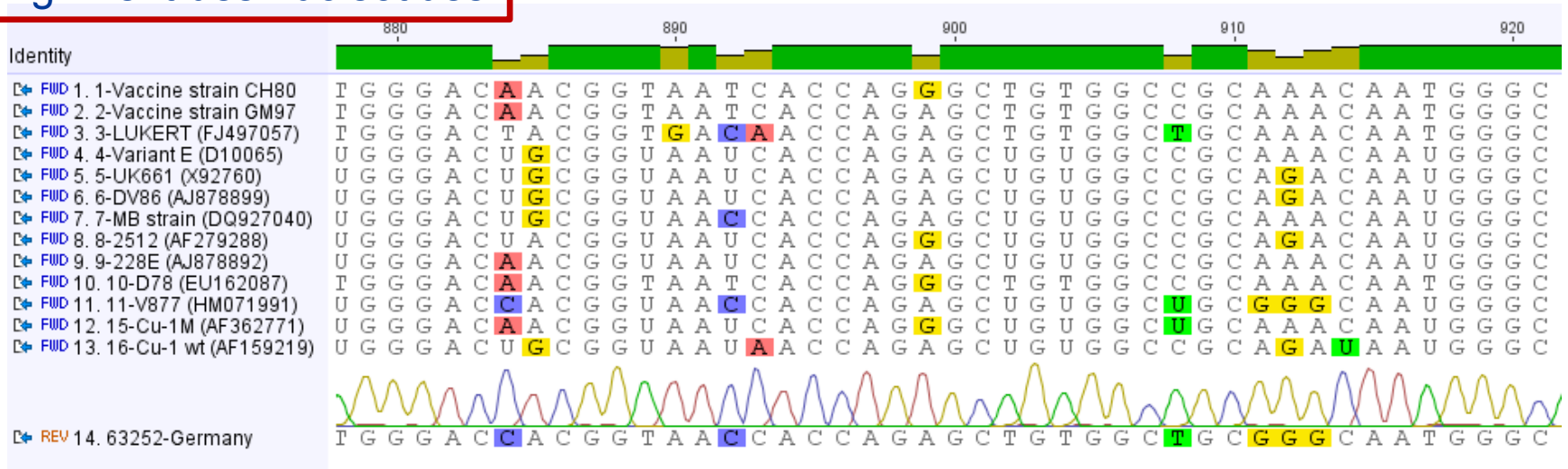
Séquençage du génome et phylogénie

Donne plus d'information génétique et détecte le réassortiment des IBDVs

Typage IBDV

Alignment des nucléotides

Nucleotide sequence. Partial VP2 gene.



Identité des nucléotides / Comparaison

	1-Vaccine str…	2-Vaccine str…	3-LUKERT (F…	4-Variant E (…	5-UK661 (X9…	6-DV86 (AJ8…	7-MB strain (…	8-2512 (AF2…	9-228E (AJ8…	10-D78 (EU1…	11-V877 (HM…	15-Cu-1M (A…	16-Cu-1 wt (…	63252-Germ…
1-Vaccine strain CH80		96.9%	94.5%	95.2%	93.3%	93.6%	95.3%	96.6%	96.9%	100%	88.8%	99.3%	95.8%	88.5%
2-Vaccine strain GM97	96.9%		95.0%	95.7%	93.8%	94.1%	94.5%	96.0%	99.8%	96.8%	89.2%	96.0%	95.8%	88.7%
3-LUKERT (FJ497057)	94.5%	95.0%		94.4%	91.7%	92.5%	93.2%	94.5%	95.2%	94.5%	88.8%	94.3%	94.7%	88.6%
4-Variant E (D10065)	95.2%	95.7%	94.4%		93.5%	93.7%	93.9%	95.8%	95.6%	95.5%	88.1%	95.0%	96.0%	88.5%
5-UK661 (X92760)	93.3%	93.8%	91.7%	93.5%		99.4%	98.4%	94.1%	93.9%	93.6%	86.9%	93.1%	94.1%	87.5%
6-DV86 (AJ878899)	93.6%	94.1%	92.5%	93.7%	99.4%		99.0%	94.7%	94.2%	93.8%	87.0%	93.4%	94.7%	87.2%
7-MB strain (DQ927040)	95.3%	94.5%	93.2%	93.9%	98.4%	99.0%		94.9%	94.4%	95.4%	88.0%	95.7%	94.1%	88.3%
8-2512 (AF279288)	96.6%	96.0%	94.5%	95.8%	94.1%	94.7%	94.9%		96.1%	96.8%	89.0%	96.7%	96.9%	89.6%
9-228E (AJ878892)	96.9%	99.8%	95.2%	95.6%	93.9%	94.2%	94.4%	96.1%		96.9%	89.2%	96.3%	95.5%	89.0%
10-D78 (EU162087)	100%	96.8%	94.5%	95.5%	93.6%	93.8%	95.4%	96.8%	96.9%		88.8%	99.5%	95.9%	88.7%
11-V877 (HM071991)	88.8%	89.2%	88.8%	88.1%	86.9%	87.0%	88.0%	89.0%	89.2%	88.8%		88.6%	89.0%	100%
15-Cu-1M (AF362771)	99.3%	96.0%	94.3%	95.0%	93.1%	93.4%	95.7%	96.7%	96.3%	99.5%	88.6%		95.3%	89.6%
16-Cu-1 wt (AF159219)	95.8%	95.8%	94.7%	96.0%	94.1%	94.7%	94.1%	96.9%	95.5%	95.9%	89.0%	95.3%		88.8%
63252-Germany (reversed)	88.5%	88.7%	88.6%	88.5%	87.5%	87.2%	88.3%	89.6%	89.0%	88.7%	100%	89.6%	88.8%	

Typage des souches IBDV (classic, vvIBDV, vaccins, variant).

Typage IBDV

Recherche dans les bases de données publiques (GenBank)
Maximum de comparaison et d'identité avec d'autres souches « Publication »
BLAST.

Name	% Pair	Sequence Length	Description	Topology	Molecule Type
HM071991	100.0%	423	Infectious bursal disease virus strain V877-W polyprotein gene, partial cds	linear	DNA
EU544160	99.8%	403	Infectious bursal disease virus strain vaccine Poulvac-Bursa-F VP2 mRNA, partial cds	linear	DNA
AJ586968	99.7%	399	Infectious bursa disease virus partial VP2 gene, genomic RNA, isolate V877 (vaccine)	linear	DNA
AF148075	99.7%	390	Infectious bursal disease virus isolate Bursavac live segment A VP2 protein mRNA, partial cds	linear	DNA
AF069577	99.7%	381	Infectious bursal disease virus strain V877 polyprotein VP2 hypervariable region mRNA, partial ...	linear	DNA
HM071990	99.3%	423	Infectious bursal disease virus strain V877-V polyprotein gene, partial cds	linear	DNA
AJ878882	99.2%	483	Infectious bursal disease virus partial VP2 gene for outer capsid protein, genomic RNA, strain ...	linear	DNA
AF148074	99.0%	390	Infectious bursal disease virus isolate V877/K segment A VP2 protein mRNA, partial cds	linear	DNA
AF148073	99.0%	390	Infectious bursal disease virus isolate 002/73 segment A VP2 protein mRNA, partial cds	linear	DNA
AF069578	99.0%	381	Infectious bursal disease virus strain V877/K polyprotein VP2 hypervariable region mRNA, parti...	linear	DNA
X03993	98.9%	537	Infectious bursal disease virus gene for polyprotein	linear	DNA
AJ878883	98.8%	483	Infectious bursal disease virus partial VP2 gene for outer capsid protein, genomic RNA, strain ...	linear	DNA
AF069579	98.4%	380	Infectious bursal disease virus strain GT101 polyprotein VP2 hypervariable region mRNA, parti...	linear	DNA
AF148080	97.9%	425	Infectious bursal disease virus isolate 06/95 VP2 protein gene, partial cds	linear	DNA
AF381000	97.9%	425	Infectious bursal disease virus isolate 01/96 VP2 protein gene, partial cds	linear	DNA
HM071998	97.9%	423	Infectious bursal disease virus strain 1127-1 polyprotein gene, partial cds	linear	DNA
AF381003	97.7%	390	Infectious bursal disease virus isolate R-1 VP2 protein mRNA, partial cds	linear	DNA
AF381004	97.6%	425	Infectious bursal disease virus isolate SS-1 VP2 protein gene, partial cds	linear	DNA
AF426065	97.4%	390	Infectious bursal disease virus isolate 01/01 polyprotein gene, partial cds	linear	DNA
AJ878908	97.3%	483	Infectious bursal disease virus partial VP2 gene for outer capsid protein, genomic RNA, strain ...	linear	DNA
AF381007	97.2%	390	Infectious bursal disease virus isolate T-4 VP2 protein mRNA, partial cds	linear	DNA
AF381002	97.2%	425	Infectious bursal disease virus isolate M-1 VP2 protein gene, partial cds	linear	DNA
HM071999	97.2%	423	Infectious bursal disease virus strain 1004-3 polyprotein gene, partial cds	linear	DNA
HM071997	97.2%	423	Infectious bursal disease virus strain 997-13 polyprotein gene, partial cds	linear	DNA
HM071995	97.2%	423	Infectious bursal disease virus strain 997-6 polyprotein gene, partial cds	linear	DNA
AF381006	96.9%	425	Infectious bursal disease virus isolate K-2 VP2 protein gene, partial cds	linear	DNA

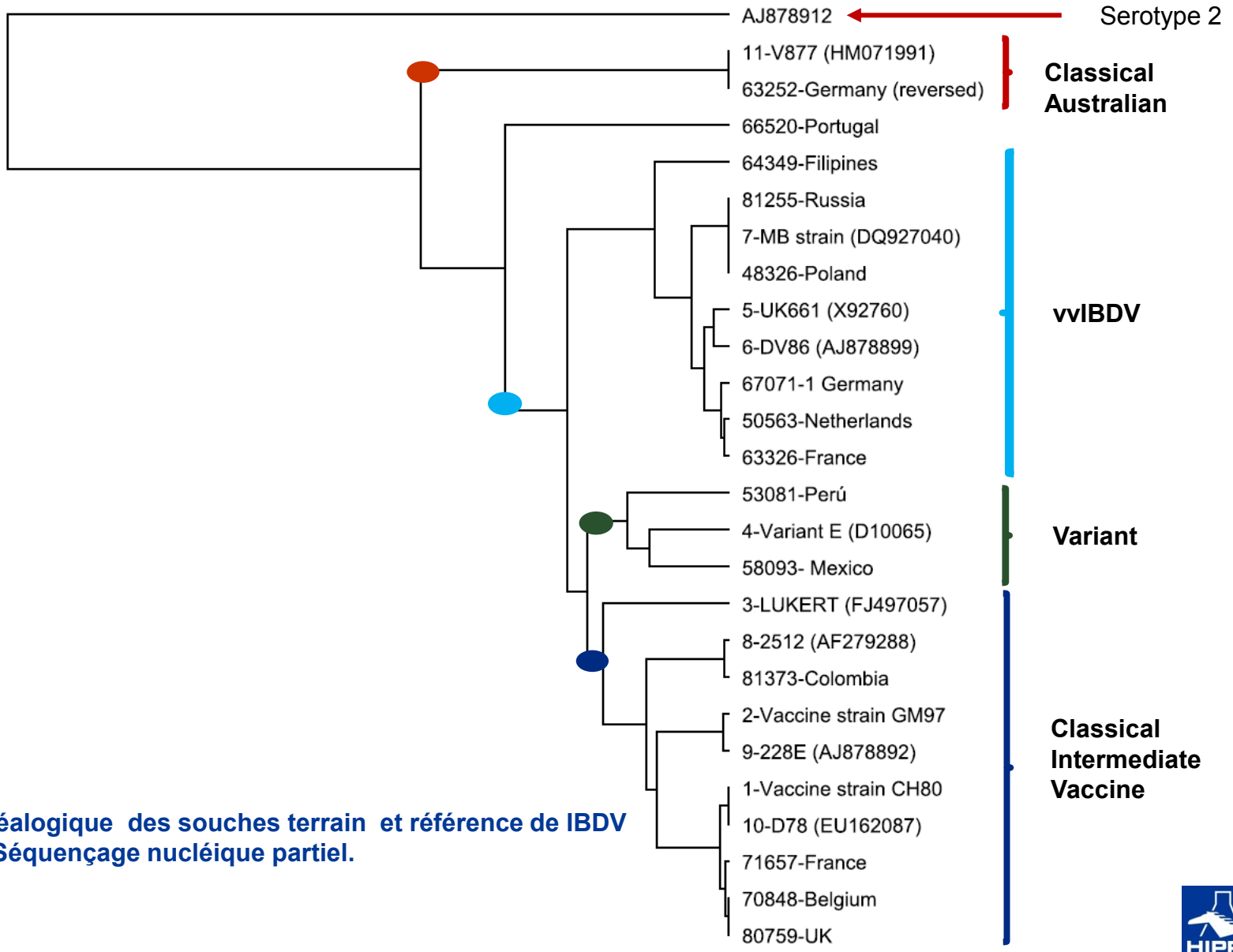
Typage IBDV

Quelques échantillons reçu ds notre labo…… du monde entier

Identité des Nucléotides parmi le séquençage partiel du gène VP2 obtenu des souches terrains et souches de références.

	48326-Poland	50563-Netherlands	53081-Perú	58093- Mexico	63252-Germany	63326-France	64349-Filipines	66520-Portugal	67071-1 Germany	70848-Belgium	71657-France	80759-UK	81255-Russia	81373-Colo
1-Vaccine strain CH80	93.9	93.5	92.9	92.1	88.5	93.7	92.1	90.6	93.2	99.5	99.5	99.6	94.1	96.2
2-Vaccine strain GM97	94.2	94.0	92.9	92.3	88.7	94.1	92.1	90.6	93.4	96.8	96.8	96.9	94.4	95.8
3-LUKERT (FJ497057)	93.1	92.2	92.3	92.3	88.6	92.2	90.2	90.6	91.5	94.4	94.9	94.7	93.1	94.5
4-Variant E (D10065)	94.1	92.9	95.7	96.6	88.5	93.1	91.3	91.7	92.9	95.5	95.8	95.9	93.9	95.8
5-UK661 (X92760)	98.3	98.6	91.4	90.5	87.5	98.8	96.5	90.3	98.6	93.7	93.8	94.1	98.4	94.1

Typage IBDV: Arbre Généalogique



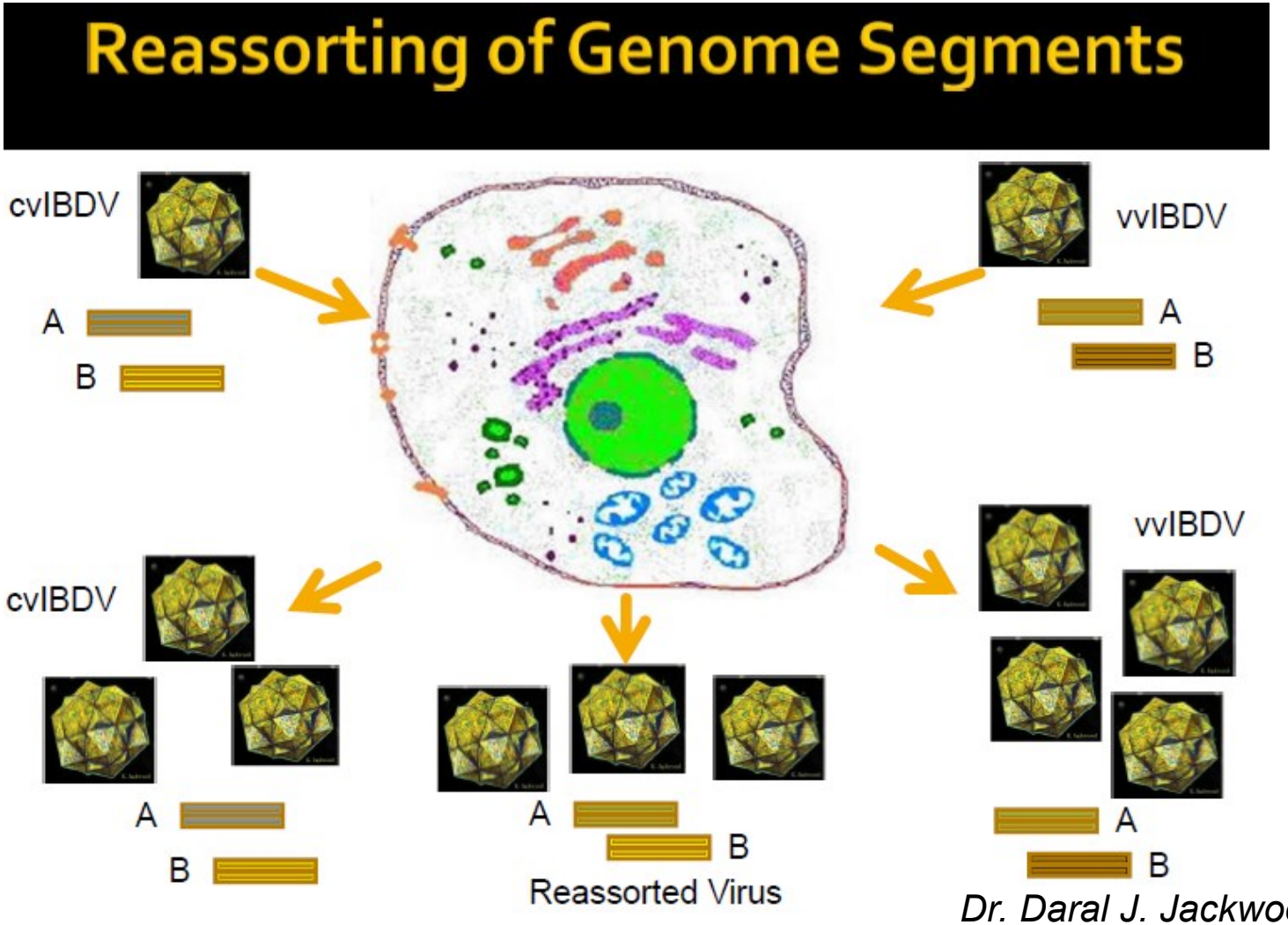
L'arbre généalogique des souches terrain et référence de IBDV
Gène VP2. Séquençage nucléique partiel.

GumboCheck en Afrique du Sud

% d'identité nucléique entre les souches IBDV terrain et souche de références.
Gène VP2. Séquençages nucléique partiel

	48158	48159	49639	49640	50971	50972	50973-2	50973	61180	Classical	Vaccine A	Vaccine B	Variant	vvIBDV
48158 SudAfrica		100.0	91.4	100.0	94.7	94.7	94.5	94.3	96.4	93.2	94.0	94.4	93.9	98.4
48159 SudAfrica	100.0		91.3	100.0	94.7	94.7	94.5	94.3	96.4	93.2	93.9	94.4	93.9	98.4
49639-SudAfrica	91.4	91.3		90.7	88.5	88.5	92.6	92.3	89.8	91.6	91.9	91.9	95.6	90.3
49640-SudAfrica	100.0	100.0	90.7		95.0	95.0	94.4	94.2	96.3	93.1	94.0	94.2	93.9	98.4
50971-SudAfrica	94.7	94.7	88.5	95.0		99.6	91.1	91.2	93.6	90.2	91.3	91.7	91.3	95.0
50972-SudAfrica	94.7	94.7	88.5	95.0	99.6		90.7	90.8	93.6	89.7	91.3	91.3	90.9	95.0
50973-2-SudAfrica	94.5	94.5	92.6	94.4	91.1	90.7		99.8	93.6	94.5	96.2	96.0	95.8	94.1
50973-SudAfrica	94.3	94.3	92.3	94.2	91.2	90.8	99.8		93.4	94.3	96.0	95.5	95.6	93.9
61180-SudAfrica	96.4	96.4	89.8	96.3	93.6	93.6	93.6	93.4		90.4	92.2	92.0	92.5	96.2
Classical strain	93.2	93.2	91.6	93.1	90.2	89.7	94.5	94.3	90.4		94.5	95.0	94.4	91.7
Vaccine A	94.0	93.9	91.9	94.0	91.3	91.3	96.2	96.0	92.2	94.5		96.9	95.2	93.3
Vaccine B	94.4	94.4	91.9	94.2	91.7	91.3	96.0	95.5	92.0	95.0	96.9		95.7	93.8
Variant strain	93.9	93.9	95.6	93.9	91.3	90.9	95.8	95.6	92.5	94.4	95.2	95.7		93.5
vvIBDV strain	98.4	98.4	90.3	98.4	95.0	95.0	94.1	93.9	96.2	91.7	93.3	93.8	93.5	

Future…..



Dr. Daral J. Jackwood

Future....

Naturally Reassorted vvIBDV

- Reassorted vvIBDV do not cause high mortality.
- Genome segment B is partially responsible for virulence.
- Molecular diagnosis of vvIBDV requires identification of genome segment B.

Dr. Daral J. Jackwood

Outils moléculaires: Contrôle de la vaccination

- **Confirmer l' absence de virus terrain dans la bourse avant la vaccination:**
  - **Situation épidémiologique**
  - **Système de biosécurité**
- **Confirmer la présence du virus vaccinale après la vaccination:**
  - **Qualité de la vaccination**
  - **Choix du vaccin adéquat**

situation	Avant la vaccination	7 jours aprèsla vaccination
1	-	-
2	-	Virus vaccinale
3	Virus terrain	Virus vaccinale
4	Virus terrain	Virus terrain

Conclusions

- La combinaison de différentes techniques de laboratoire est nécessaire pour le diagnostique et le contrôle du challenge des IBDV.

- Les techniques moléculaires permettent une détection rapide, sensitive des IBDV en plus du typage des souches a l'échelle mondiale.

- Peuvent être utilisées pour:
  - Diagnostic, surtout pour les formes sub-clinique et immunosuppressive
  - Typage du type de virus: cvIBDV, vvIBDV, Variant
  - Evaluation de la vaccination: témoin de prise vaccinale
  - Evaluation des procédures de nettoyage et désinfection

- Quoique….ces **techniques doivent être adaptées continuellement pour pouvoir tjrs détecter les nouveaux réassortiments des souches d' IBDV.**

Merci!