

Combating Unknown Enemies with the Help of Molecular Diagnostics

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11th February 2025

Exhibition Date:
12th, 13th, 14th
February 2025



We cordially invite you at

NOVA CON

Conference on InNOVation

Venue: Biswa Bangla Convention Centre
New Town, Action Area-1, Kolkata, West Bengal 700156

International Conference

on

POULTRY BUSINESS

On the eve of
11th KOLKATA
INTERNATIONAL
POULTRY
FAIR 2025

Metagenomics

NGS (Next Generation Sequencing)

RNA sequencing is key to diagnostics

RNA is produced by all viruses and bacteria

Total random RNA sequencing does not require a hypothesis

Sequencing provides unsurpassed specificity

Proportion of reads may provide a “semi quantitative” representation

If random, it provides a representation of “all” agents present

May detect mixed infections that are relevant to clinical diagnosis

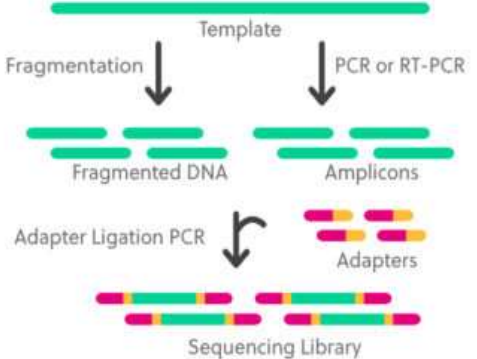


NGS (Next Generation Sequencing)

STEP 1:
Extraction



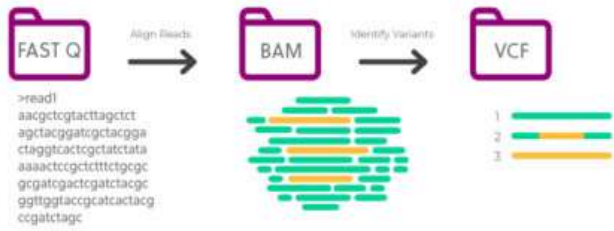
STEP 2:
Library
Prep



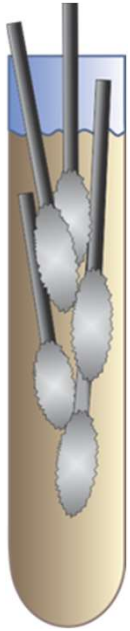
STEP 3:
Sequencing



STEP 4:
Analysis



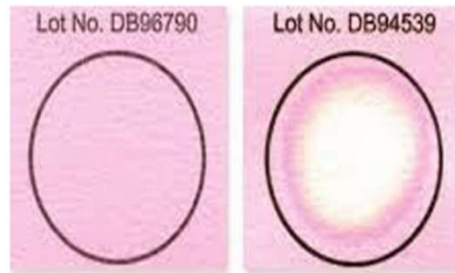
Manual for sample collection



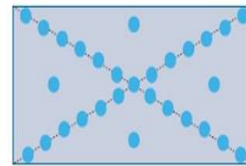
A



B

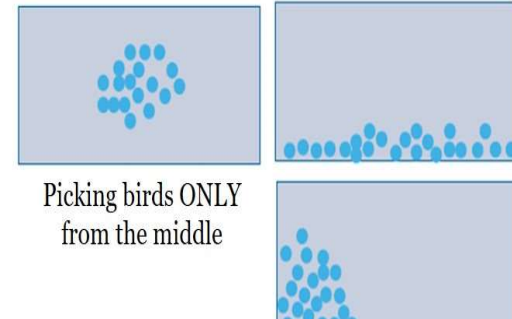


Recommended method



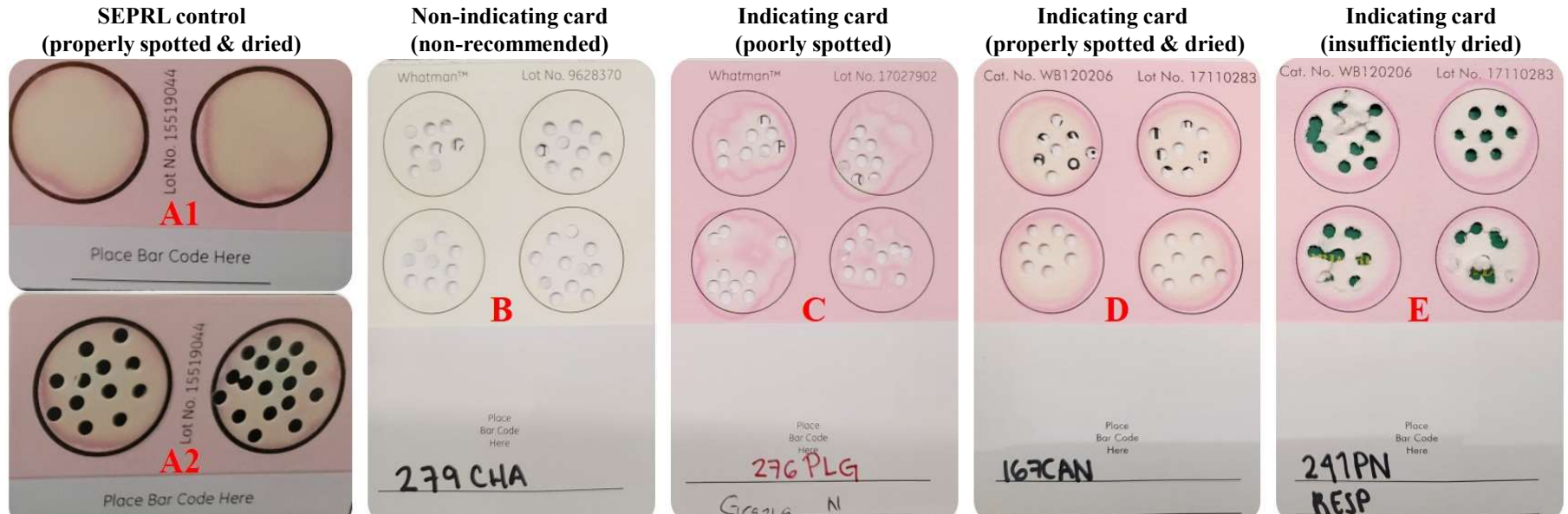
Select the birds randomly

Non-recommended method



Picking birds ONLY from the middle

Manual for sample collection



QC of FTA cards. These are Among the key aspects to pay attention to during sample preparation in the field. Panels A and D illustrates FTA cards (from SEPRL control sample and BAH field sample, respectively) that are properly spotted (with the recommended volumes of samples, i.e., approximately 125 μ L) and sufficiently dried. Panel B illustrates non-indicating FTA card in which it is difficult to locate where the samples were spotted or verify if sufficient sample volumes were spotted. Panel E shows an insufficiently dried (damp) FTA card spotted with a field sample, which are difficult to punch out discs for subsequent extraction of nucleic acids.

Manual for sample collection



Bursa sampling



Spleen sampling



1

1.1 Evenly distributed sampling all over the barn

1.2 Sample 10 animals (1 swab per animal)

2

2.1 Prepare a clean workspace

2.2 Swirl swab 1 in medium

2.3 Squeeze and remove swab

2.4 Disposal of the swab

2.5 Proceed like this with remaining 9 swabs

3

3.1 Place protective layer between the back cover and the filter paper

3.2 Transfer inoculated medium to AriCard

3.3 Let dry at room temperature and remove protective layer

3.4 Write sample ID on label

3.5 Stick label to AriCard

AN-007-2021-001_Englis002

Metagenomics



Broiler flock with respiratory clinical signs, increased mortality, some with nervous signs. The Vet in charge is convinced this is a VVND case and is assuming the mortality is associated with his vaccination program vs ND. He is thinking on changing the vaccination program vs ND.

To unveil hidden pathogens:

BB Pipeline Report

[Summary](#)
[Taxonomics](#)
[Agents](#)
[De Novo](#)
[Quality Control](#)

Pipeline NGS untargeted discovery (avian)

Client BIAH

Sample 3154

Project May 2022

Description Sample ID: 043NA; Sampling Date: 2022-04-27; Origin: Mexico; Tissue: choanal/lung; Flock: broiler; Signs: apparently healthy; Suspected Agent: none

Molecule RNA

Method Extraction: MagMAX™ Viral RNA Isolation; Pre-treatment: RNaseH host rRNA depletion; Library Prep: SISPA/Nextera Flex

Generated 2022-05-17 15:02:17

Pipeline Version 0.040

Platform illumina

PLEASE NOTE: The results presented in this report are for INFORMATIONAL PURPOSES ONLY. They do not constitute diagnostic data and cannot be used to satisfy regulatory requirements. No guarantee is made as to the presence or absence of any biological agent based on the information contained in this report. BASE2BIO LLC assumes no responsibility for any harm caused by mis-use or mis-application of the information contained herein.

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Quick Links

Untargeted Taxonomics
 Tabular summary of all taxa detected above threshold, not limited to only pathogens

Viruses
Bacteria
Other

MetaView
 Interactive visualization of the taxonomic content of the sample

Targeted Results
 Agent-specific phylogenies and genotype tests (not available for all taxa)

De Novo
 Details of *de novo* assembly, including *least common ancestor* (LCA) assignments for all contigs and access to the raw sequences

Viruses
Bacteria
Other

Overview of Results

Detected Taxa of Interest What's this?

Name	Estimated count	Specific count	Relative abundance	Group	Detected by	Confidence
Avian metapneumovirus	31316	31306	17%	A	Read classification/ <i>de novo</i> assembly	high
Bordetella avium	17382	12286	9.4%	–	Read classification/ <i>de novo</i> assembly	high
Influenza A virus	2067	2066	1.1%	H5/N2	Read classification/ <i>de novo</i> assembly	high
Avian orthoavulavirus 1	804	803	0.43%	V.2	Read classification/ <i>de novo</i> assembly	high
Streptococcus pluranimalium	644	34	0.35%	–	Read classification/ <i>de novo</i> assembly	medium
Enterococcus cecorum	178	13	0.096%	–	Read classification/ <i>de novo</i> assembly	medium
Avian leukosis virus	57	48	0.031%	–	Read classification	medium

Positive Genotype Assays

Test ID	Description	Locus	Result
2560319/F_cleavage	NDV cleavage site - determinant of virulence	fusion protein	positive

Run QC Summary

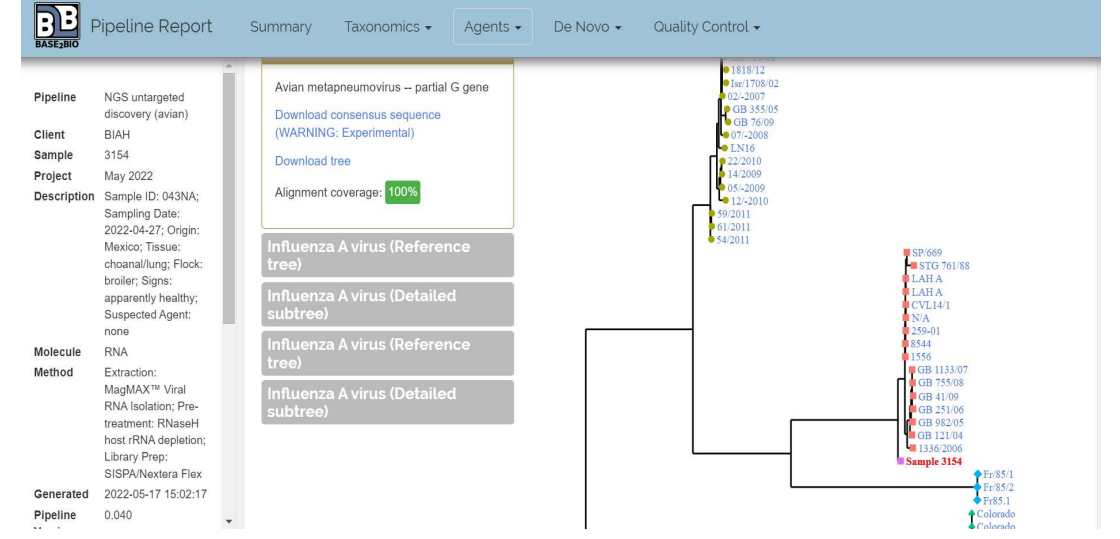
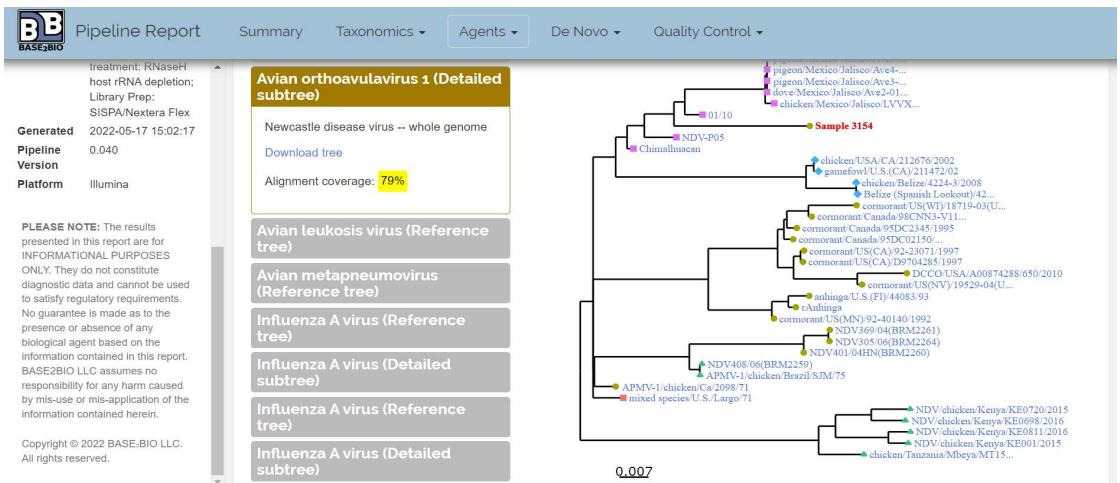
Metric	Value	Status
Read mapping quality filter	87.8%	pass

Metagenomics

To unveil hidden pathogens:



Some nervous signs
Torticollis caused by SHS



Metagenomics

To unveil hidden pathogens:



Broiler flock which experienced a curve of mortality starting the second week of age. Lesions in liver, kidneys and diarrhea are the main findings. Vet is suspecting on Malabsorption syndrome caused by Reovirus and is asking to include the Reo fraction vs malabsorption in the breeders.

BB BASE2BIO

Pipeline Report
Summary
Taxonomics ▾
Agents ▾
De Novo ▾
Quality Control ▾

Pipeline NGS untargeted discovery (avian)

Client BIAH

Sample 3112

Project May 2022

Description Sample ID: 001BTC; Sampling Date: 2022-01-13; Origin: Mexico; Tissue: Spleen/bursa; Flock: broiler; Signs: apparently healthy; Suspected Agent: none

Molecule RNA

Method Extraction: MagMAX™ Viral RNA Isolation; Pre-treatment: RNaseH host rRNA depletion; Library Prep: SISPA/Nextera Flex

Generated 2022-05-17 14:10:50

Pipeline 0.040

Quick Links

Untargeted Taxonomics

Tabular summary of all taxa detected above threshold, not limited to only pathogens

Viruses
Bacteria
Other

MetaView

Interactive visualization of the taxonomic content of the sample

Targeted Results

Agent-specific phylogenies and genotype tests (not available for all taxa)

Phylogeny
Genotype Tests

Overview of Results

Detected Taxa of Interest What's this?

Name	Estimated count	Specific count	Relative abundance	Group	Detected by	Confidence
Avastrovirus	563	563	1%	–	Read classification/de novo assembly	high
Enterococcus faecalis	470	35	0.84%	–	Read classification/de novo assembly	medium
Enterococcus hirae	107	8	0.19%	–	Read classification/de novo assembly	medium
Enterococcus faecium	67	5	0.12%	–	Read classification	medium

Positive Genotype Assays

Metagenomics

To measure vaccination program efficacy:



Broiler flock with normal mortality and no clinical signs. The Vet is asked to take samples for PCR testing before slaughter age. The report is positive to IBD Virus. The Vet is recommended to modify the vaccination program.

BB BASE2BIO Pipeline Report

[Summary](#)
[Taxonomics](#)
[Agents](#)
[De Novo](#)
[Quality Control](#)

Pipeline NGS untargeted discovery (avian)

Client BIAH

Sample 3114

Project May 2022

Description Sample ID: 003BTC; Sampling Date: 2022-01-13; Origin: Mexico; Tissue: Spleen/bursa; Flock: broiler; Signs: apparently healthy; Suspected Agent: none

Molecule RNA

Method Extraction: MagMAX™ Viral RNA Isolation; Pre-treatment: RNaseH host rRNA depletion; Library Prep: SISPA/Nextera Flex

Generated 2022-05-17 14:12:15

Pipeline 0.040

Quick Links

Untargeted Taxonomics

Tabular summary of all taxa detected above threshold, not limited to only pathogens

Viruses

Bacteria

Other

MetaView

Interactive visualization of the taxonomic content of the sample

Targeted Results

Agent-specific phylogenies and genotype tests (not available for all taxa)

[Phylogeny](#)

[Genotype Tests](#)

Overview of Results

Detected Taxa of Interest What's this?

Name	Estimated count	Specific count	Relative abundance	Group	Detected by	Confidence
Enterococcus cecorum	131	3	0.11%	–	Read classification	low
Infectious bursal disease virus	77	77	0.067%	–	Read classification/de novo assembly	high
Siciniavirus	72	72	0.062%	–	Read classification/de novo assembly	high
Infectious bronchitis virus	3	3	0.0026%	–	Read classification	low

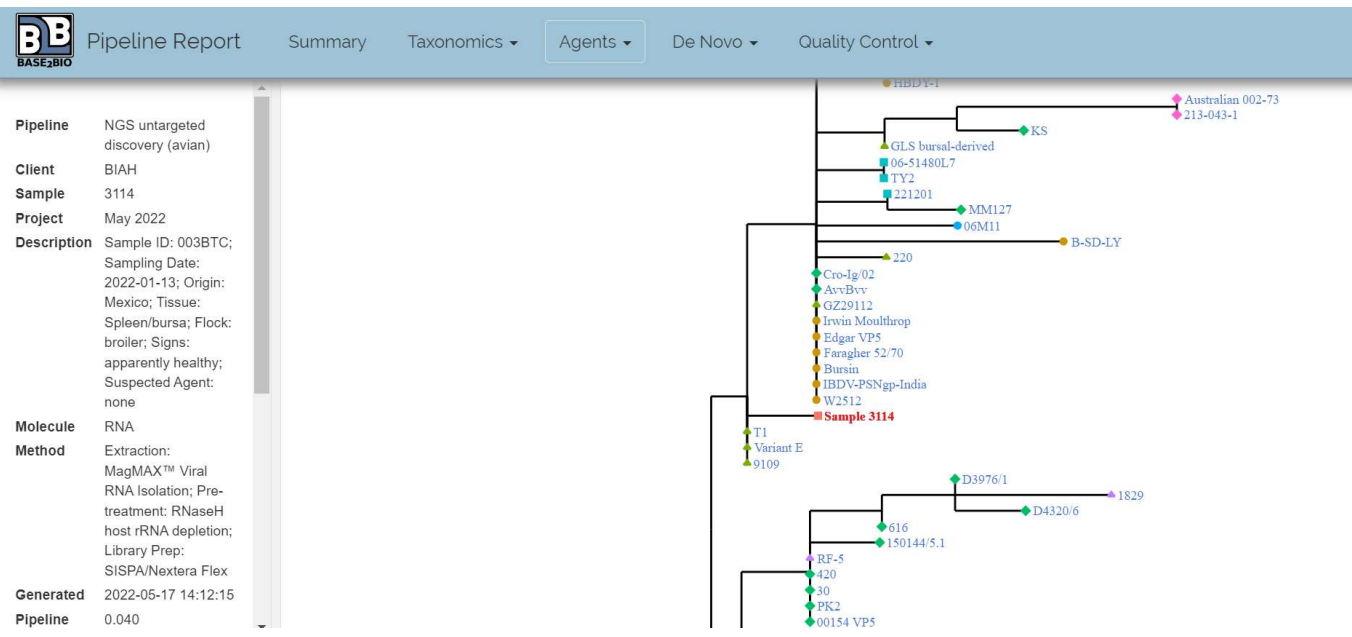
Positive Genotype Assays

Metagenomics

To measure vaccination program efficacy:



IBD variant E



Metagenomics

To detect infectious causes of immune suppression:

Causes of bursal atrophy

Not all is about IBD

CAV

Reo

Adeno

Marek

AI

VVND

Mycotoxins

Stress

The screenshot displays a 'Pipeline Report' for an NGS untargeted discovery (avian) pipeline. The report includes a sidebar with sample details, a 'Quick Links' section with 'Untargeted Taxonomics', 'MetaView', and 'Targeted Results', and an 'Overview of Results' section. The 'Detected Taxa of Interest' table lists Avian gyrovirus 2 and Avian leukosis virus. The 'Positive Genotype Assays' section shows 'None'.

Name	Estimated count	Specific count	Relative abundance	Group	Detected by	Confidence
Avian gyrovirus 2	251	251	0.051%	-	Read classification/de novo assembly	high
Avian leukosis virus	14	13	0.0029%	ALV-J	Read classification	low

Metagenomics

To investigate mixed infections:

BB Pipeline Report | Summary | Taxonomics | Agents | De Novo | Quality Control

Untargeted Taxonomics
 Tabular summary of all taxa detected above threshold, not limited to only pathogens

Viruses | Bacteria | Other

MetaView
 Interactive visualization of the taxonomic content of the sample

Targeted Results
 Agent-specific phylogenies and genotype tests (not available for all taxa)

Phylogeny | Genotype Tests

Detected Taxa of Interest *What's this?*

Name	Estimated count	Specific count	Relative abundance	Group	Detected by	Confidence
Salmonella enterica	2231	5	0.89%	-	Read classification	medium
Enterococcus cecorum	826	123	0.33%	-	Read classification/de novo assembly	high
Influenza A virus	779	779	0.31%	H5/N2	Read classification/de novo assembly	high
Enterococcus faecalis	114	17	0.045%	-	Read classification	medium
Sicivirus	61	61	0.024%	-	Read classification/de novo assembly	high
Infectious bronchitis virus	57	50	0.023%	-	Read classification	high
Chicken anemia virus	10	10	0.004%	-	Read classification/de novo assembly	medium

De Novo

Avian Influenza +
 Infectious Bronchitis
 +
 CAV
 +
 Bacteria

Metagenomics

To investigate mixed infections:



BB Pipeline Report

Summary Taxonomics Agents De Novo Quality Control

Pipeline NGS untargeted discovery (avian)

Client BIAH

Sample 3110

Project May 2022

Description Sample ID: 264PN; Sampling Date: 2021-12-07; Origin: Mexico; Tissue: choanal; Flock: broiler; Signs: apparently healthy; Suspected Agent: none

Molecule RNA

Method Extraction: MagMAX™ Viral RNA Isolation; Pre-treatment: RNaseH host rRNA depletion; Library Prep: SISPA/Nextera Flex

Generated 2022-05-17 14:09:08

Pipeline 0.040

Pipeline NGS untargeted discovery (avian)

Client BIAH

Sample 3110

Project May 2022

Description Sample ID: 264PN; Sampling Date: 2021-12-07; Origin: Mexico; Tissue: choanal; Flock: broiler; Signs: apparently healthy; Suspected Agent: none

Molecule RNA

Method Extraction: MagMAX™ Viral RNA Isolation; Pre-treatment: RNaseH host rRNA depletion; Library Prep: SISPA/Nextera Flex

Generated 2022-05-17 14:09:08

Pipeline 0.040

Quick Links

Untargeted Taxonomics
Tabular summary of all taxa detected above threshold, not limited to only pathogens

Viruses Bacteria Other

MetaView
Interactive visualization of the taxonomic content of the sample

Targeted Results
Agent-specific phylogenies and genotype tests (not available for all taxa)

Phylogeny Genotype Tests

De Novo
Details of de novo assembly, including least common ancestor (LCA) assignments for all contigs and access to the raw sequences

Viruses Bacteria Other

Overview of Results

Detected Taxa of Interest *What's this?*

Name	Estimated count	Specific count	Relative abundance	Group	Detected by	Confidence
Gallibacterium	7544	3841	4%	–	Read classification/de novo assembly	high
Avibacterium	3278	1669	1.7%	–/–	Read classification/de novo assembly	high
Ornithobacterium rhinotracheale	1594	1151	0.84%	–	Read classification/de novo assembly	high
Enterococcus cecorum	1525	1070	0.8%	–	Read classification/de novo assembly	high
Mycoplasma synoviae	175	151	0.092%	–	Read classification/de novo assembly	high
Streptococcus pluranimalium	49	21	0.026%	–	Read classification/de novo assembly	low

Positive Genotype Assays
None

Run QC Summary

Metric	Value	Status
Reads passing quality filter	86.5%	warn
Total passing read pairs	250,729	okay
Read pairs after trimming	208,771	okay
Median fragment length	199	okay
Fraction of reads classified	92.0%	okay
Fraction of class. reads from host	15.1%	okay
Fraction of class. reads from vectors	0.3%	okay

Metagenomics

To understand the epidemiology:

Pipeline Report

[Summary](#)
Taxonomics ▾
Agents ▾
De Novo ▾
Quality Control ▾

Pipeline NGS untargeted discovery (avian)

Client BIAH

Sample 3206

Project Aug 2022

Description Sample ID: 051AP; Sampling Date: 2022-08-04; Origin: MEX; Tissue: Immun. (spleen/bursa); Flock: broiler

Molecule RNA

Method Extraction: MagMAX™ Viral RNA Isolation; Pre-treatment: RNaseH host rRNA depletion; Library Prep: SISPA/Nextera Flex

Generated 2022-08-31 12:30:25

Pipeline Version 0.041


Platform Illumina

Quick Links


Untargeted Taxonomics

Tabular summary of **all** taxa detected above threshold, not limited to only pathogens


Viruses



Bacteria



Other



MetaView

Interactive visualization of the taxonomic content of the sample

Targeted Results

Agent-specific phylogenies and genotype tests (not available for all taxa)

Phylogeny

Genotype Tests

Overview of Results

Detected Taxa of Interest What's this?

Name	Estimated count	Specific count	Relative abundance	Group	Detected by	Confidence
Infectious bronchitis virus	4326	4060	3%	GI-3	Read classification	high
Enterococcus faecium	3632	2343	2.5%	–	Read classification/de novo assembly	high
Streptococcus pluranimalium	3125	1839	2.1%	–	Read classification/de novo assembly	high
Enterococcus hirae	825	532	0.56%	–	Read classification/de novo assembly	high
Enterococcus cecorum	369	238	0.25%	–	Read classification/de novo assembly	high

Metagenomics

To understand the epidemiology:



Microbiology
Resource Announcements



 | Virology | Announcement

Coding-complete genome sequence of a GI-13 infectious bronchitis virus from commercial chicken in India

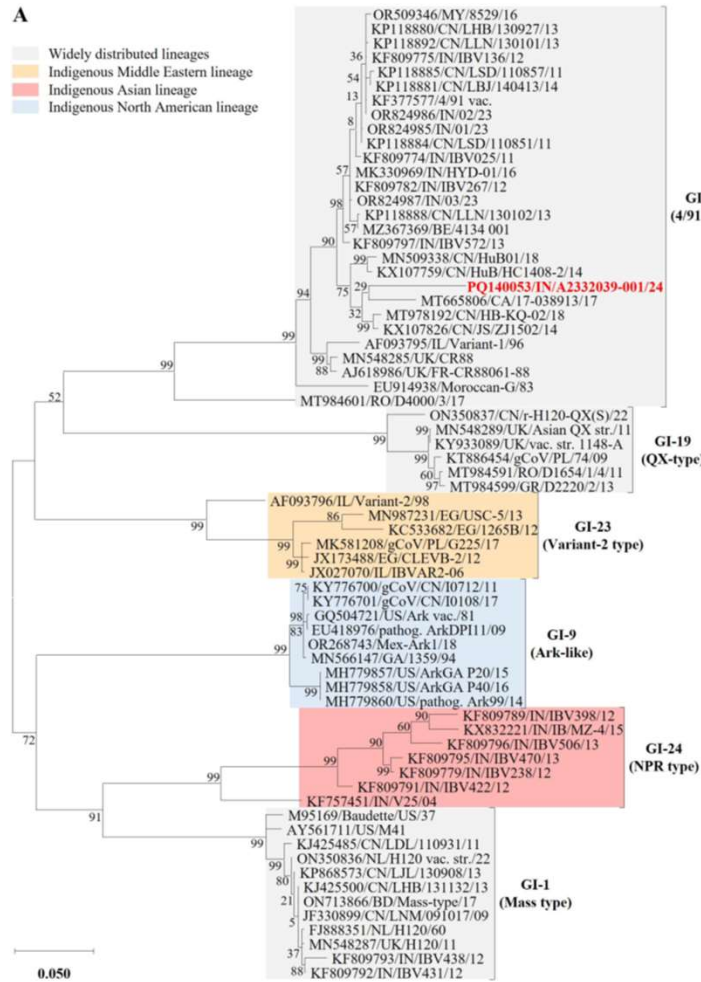
Henry M. Kariithi,¹ Jeremy D. Volkening,² Claudio L. Afonso,² Mohamed Helmy,³ Pushparaj P. Chaudhari,⁴ Eduardo L. Decanini³

AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT Infectious bronchitis virus (IBV) causes a highly contagious, acute upper respiratory disease in chickens characterized by nasal discharge, coughing, and rales. Here, the complete genome sequence of a recombinant GI-13 IBV strain ck/IN/A2332039-001/24 was sequenced from a choanal sample of a commercial broiler chicken in India using nontargeted next-generation sequencing.

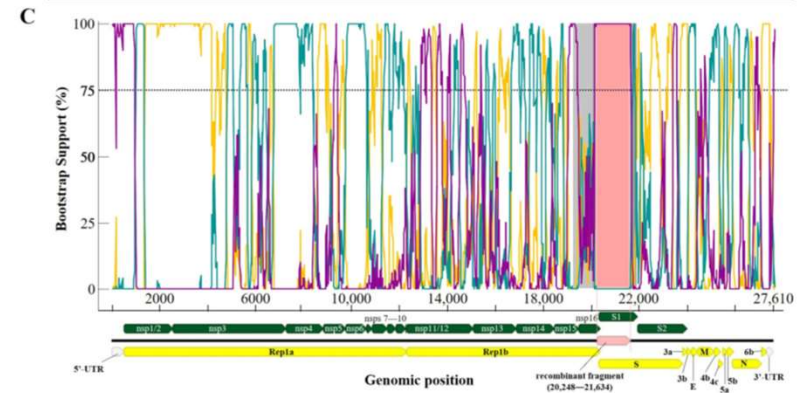
Metagenomics

To understand the epidemiology:



B

Lineage	Strain	Complete genome	Gene 1 (Rep1a)	Gene 2 (Rep1ab)	S	S1	Gene 3 (3a-3b-E)	Gene 4 (M-4b-4c)	Gene 5 (5a-5b)	Gene 6 (N)
GI-13 (4/91 type)	MT984601/RO/D4000/3/17	92.28	93.42	93.8	84.66	82.87	88.63	92.9	90.52	91.95
	MT665806/CA/17-038913/17	89.87	89.12	90.56	89.33	92.39	86.1	89.86	91.87	93.17
	KP118892/CN/LLN/130101/13	90.98	90.87	91.52	89.73	93.57	86.41	88.52	92.1	90.98
	OR824986/IN/02/23	91.09	90.9	91.53	89.81	93.69	86.41	90.72	92.1	90.98
	OR824985/IN/01/23	91.09	90.89	91.53	89.79	93.57	86.41	90.81	92.1	90.89
	KF809775/IN/IBV136/12	-	-	-	-	93.63	-	-	-	-
	KF809797/IN/IBV572/13	-	-	-	-	93.57	-	-	-	-
	KF809774/IN/IBV025/11	-	-	-	-	93.54	-	-	-	-
	MN509338/CN/HuB01/18	-	-	-	-	94.19	-	-	-	-
	KF809793/IN/IBV438/12	-	-	-	-	77.08	-	-	-	-
GI-1	KF809792/IN/IBV431/12	-	-	-	-	78.42	-	-	-	-
	MG763935/IN/53/13	91.94	93.43	93.97	82.17	78.42	86.73	92.05	95.37	93.82
	KJ425485/CN/LDL/110931/11	92.08	93.42	93.96	82.75	78.42	89.89	90.72	95.94	93.5
	ON350836/NL/H120 vaccine/22	92.19	93.38	93.93	82.2	78.54	86.73	92.63	95.49	94.39
GI-9	MN548287/UK/H120/11	91.93	93.44	93.98	82.15	78.35	86.73	92.63	95.49	93.74
	GG504720/US/Ark DPI/81	91.65	94.08	93.42	82.11	77.63	91	90.62	95.71	93.25
	MH779858/US/ArkGA P40/16	89.71	89.82	90.79	81.68	77.33	89.73	90.62	95.71	93.33
	MH779860/US/Ark99/14	89.72	89.83	90.79	81.71	77.33	89.73	90.72	95.71	93.33
	KY776700/gCoV/CN/I0712/11	91.78	94	93.52	82.39	77.76	91.15	90.62	95.49	93.25



Plot legend

- 95-99% breakpoint confidence intervals
- Track sequence with a recombinant origin
- Plot of major parent (GG504720/Ark DPI/81 {GI-9}) & rec. str. PQ140053/IN/A2332039-001/2024 {GI-13}
- Plot of minor parent (KP118892/LLN/130101/13 {GI-13}) & rec. str. PQ140053/IN/A2332039-001/2024 {GI-13}
- Plot of major parent (GG504720/Ark DPI/81 {GI-9}) & minor parent (KP118892/CN/LLN/130101/13 {GI-13})

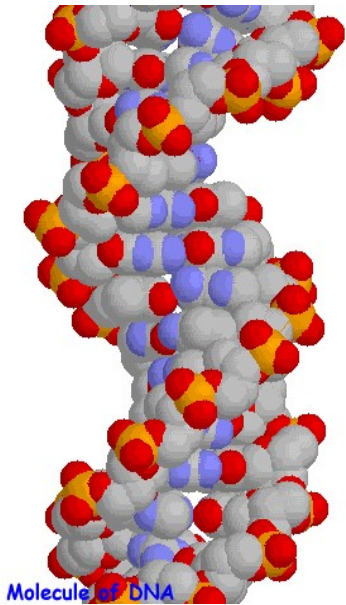
Recombination event confirmation

algorithm	# seqs detected in	p-value
RDP	1	1.068 x 10 ⁻⁷⁴
GENECOV	1	2.460 x 10 ⁻⁹³
BootScan	1	1.923 x 10 ⁻¹⁸
MaxChi	1	9.226 x 10 ⁻²⁶
Chimaera	1	7.433 x 10 ⁻²¹
SiScan	1	8.741 x 10 ⁻³⁷
3Seq	1	3.799 x 10 ⁻¹³

Targeted PCR & Sequencing

PCR (Polymerase Chain Reaction)

Gene are on nucleic acid chains (DNA, RNA)



©Roehampton Experimental Station, 1997, 1998

DNA

DNA viruses (Herpesvirus, Adenovirus, ...)

Bacteria (plasmids, nucleus)

Eucaryotes (nucleus)

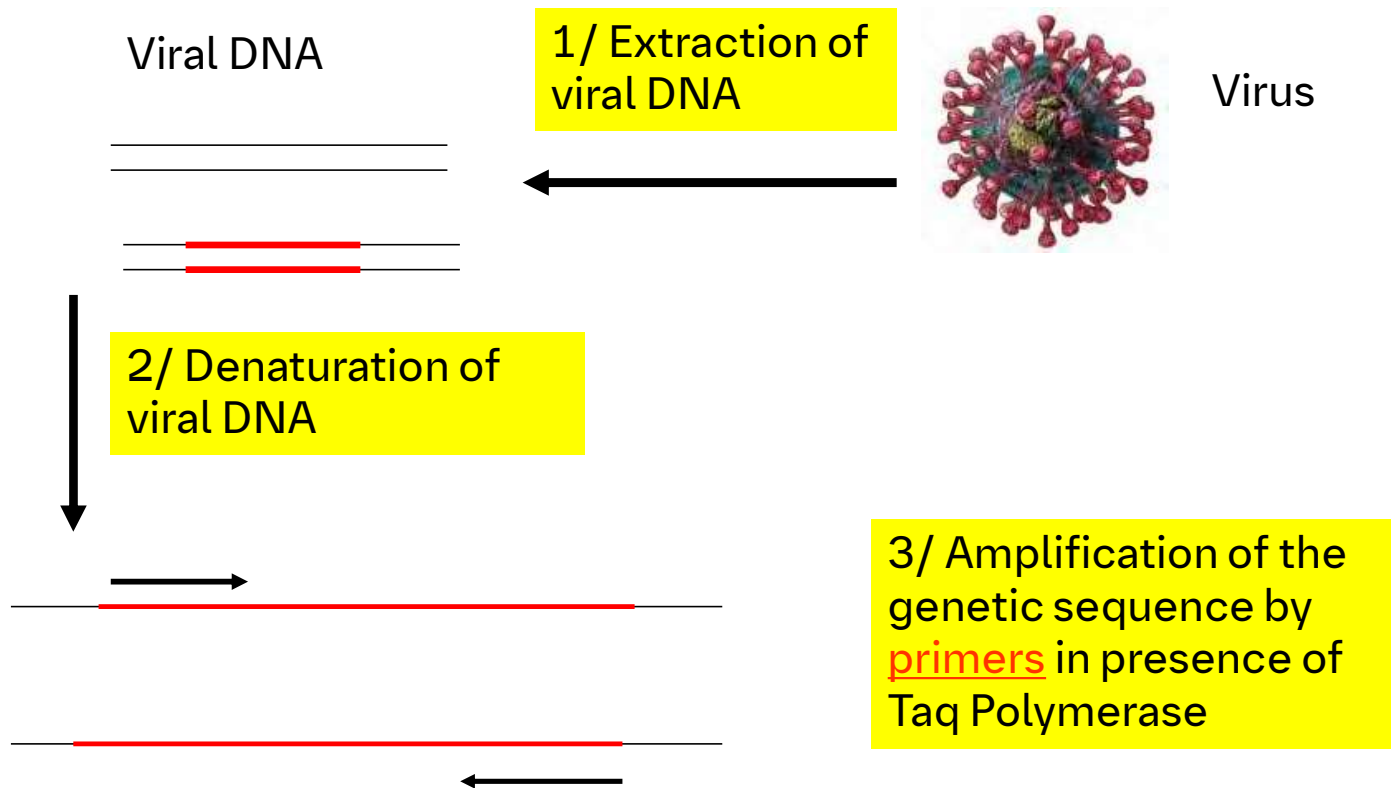
RNA

RNA viruses (Ortho- & Para-myxovirus, Metapneumovirus, ...)

Bacteria

Eucaryotes: RNAm, RNAt, RNAr

PCR (Polymerase Chain Reaction)



PCR (Polymerase Chain Reaction)

The «billion» amplified genes are then detectable using two types of PCR

Conventional PCR

Amplification then reveal by Electrophoresis

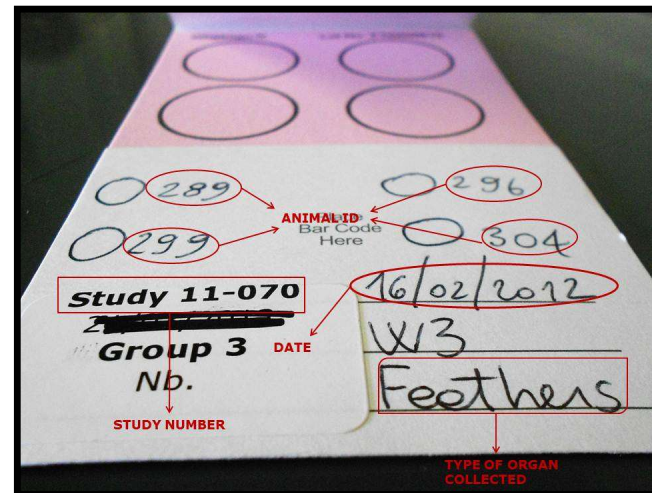
Real – Time PCR

Amplification and reveal simultaneously by Fluorescence reads

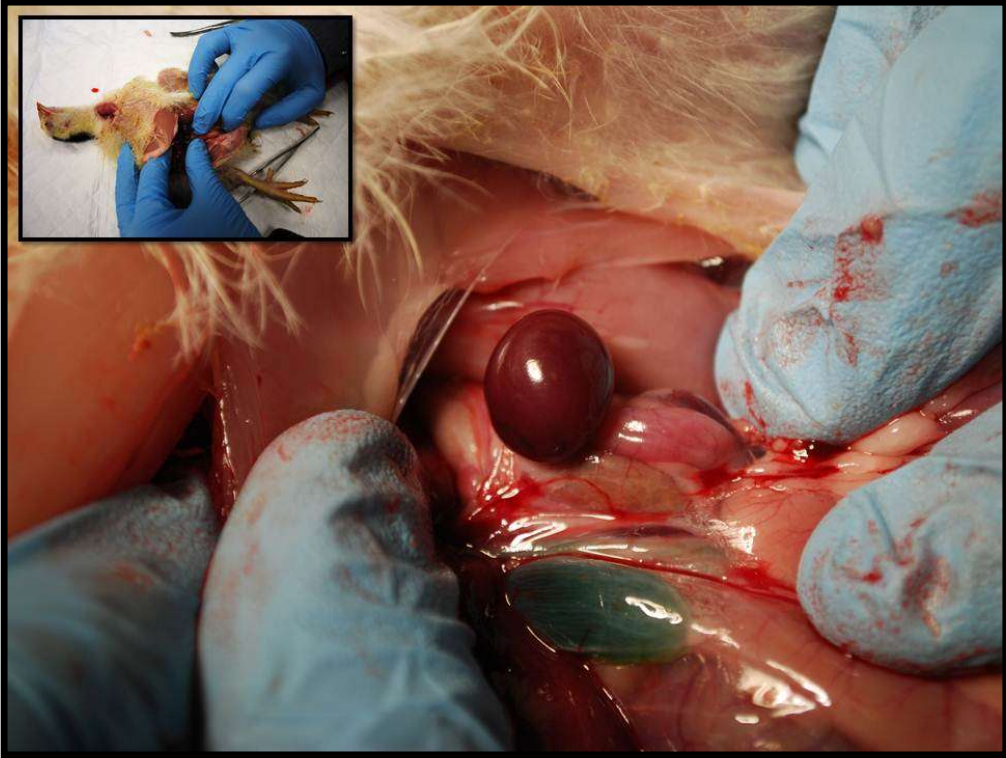
Manual for sample collection



FTA cards

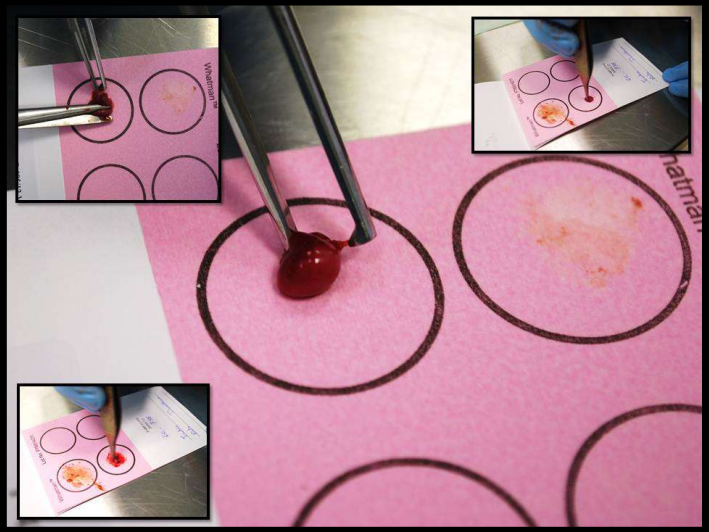


Manual for sample collection



FTA cards

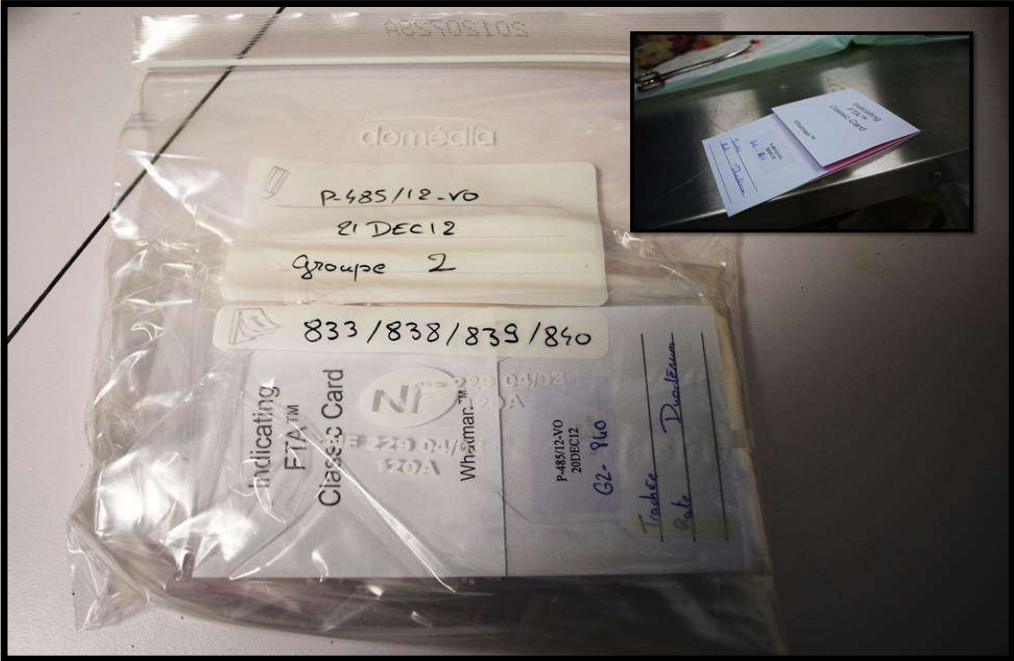
Manual for sample collection



FTA cards



Manual for sample collection



FTA cards

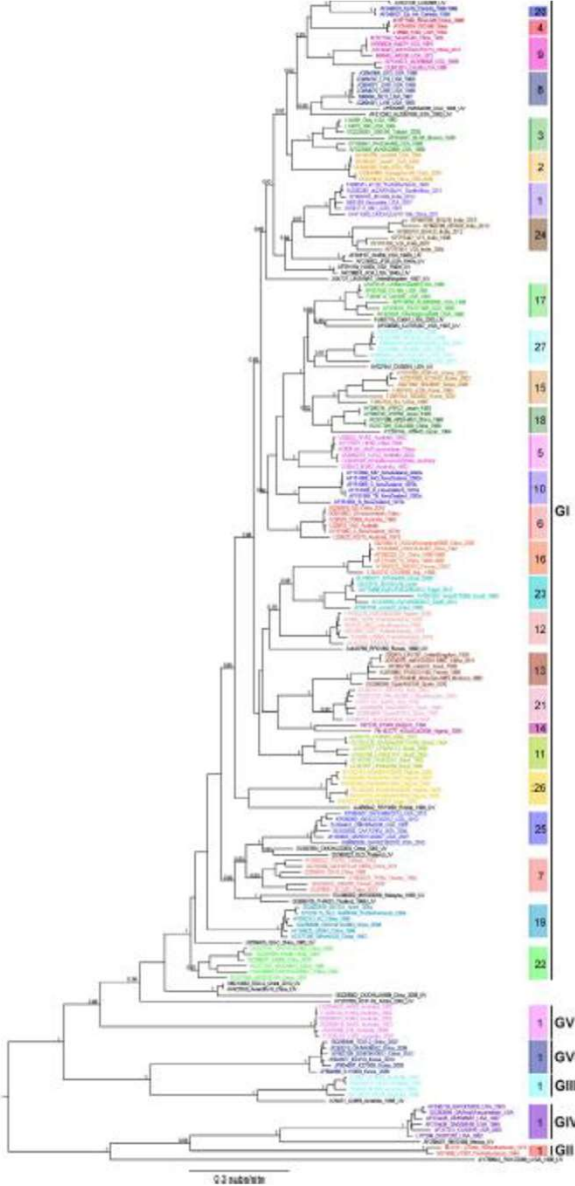
Infectious Bronchitis

Genotypes GI – GVI (nowadays GIX or GX)

Differentiation into lineages:

GI: 27 lineages (e.g. GI-1: Mass; GI-13: 793b; ...)

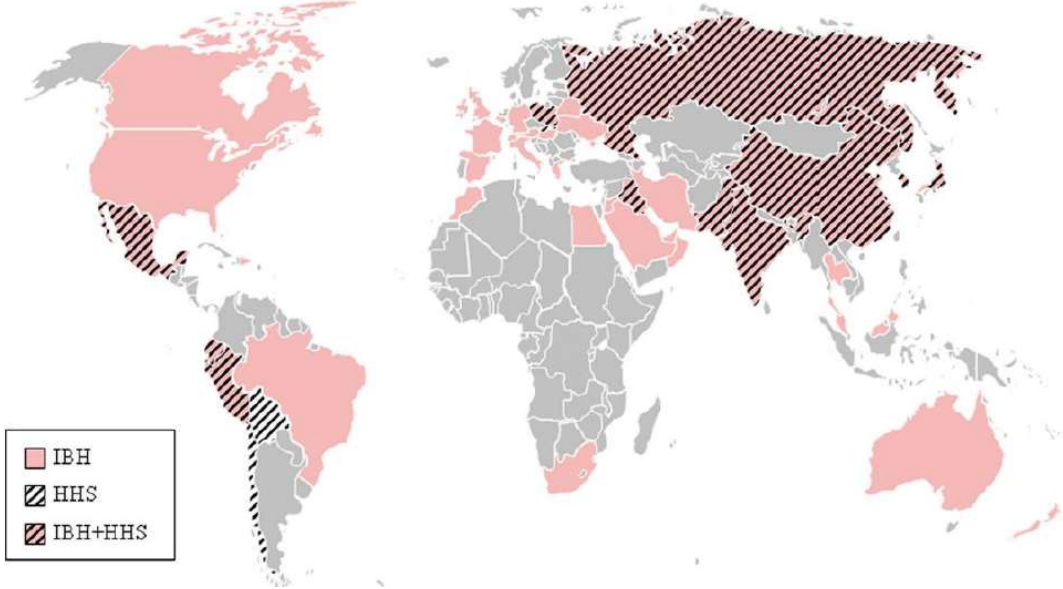
~30 lineages to date



[Valastro et Al, 2016]

Fowl Adenovirus

Disease	Mostly types
Inclusion Body Hepatitis	FAdV-2, -8a, -8b (species E) FAdV-11 (species D)
Gizzard Erosions	FAdV-1 (species A)
(Hepatitis-) Hydropericardium Syndrome	FAdV-4 (species C)



[Schachner et Al, 2018]

Fowl Adenovirus

Screening PCR

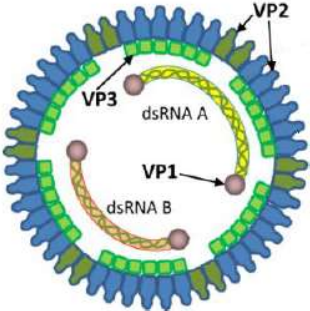
Determination of serotype by sequencing of Hexon gene



Infectious Bursal Disease

VP2 (segment A)	
A1	Classical virulent/attenuated
A2	US antigenic
A3	Very virulent
A4	dIBDV
A5	Mexican
A6	Italian
A7	Early Australian
A8	Australian variant
A9	Portugal
A0	Serotype 2



VP1 (segment B)	
B1	Classical like (incl. serotype 2)
B2	Very virulent-like
B3	Early Australian-like
B4	Polish/Tanzanian
B5	Nigerian



 Genotypes
 (e.g. A1B1, A3B2, ...)

Infectious Bursal Disease

Screening PCR

Typing PCRs (A1, A3, A7)

Partial VP1 and VP2 sequencing

IBDV directed NGS for whole VP1&VP2 sequencing

Consider recombinant vaccine strains (HVT vector vaccines or DIVA PCRs)

Marek's Disease

Meleagrid alphaherpesvirus 1 (= Herpesvirus of turkey (HVT) non-pathogenic)

Gallid alphaherpesvirus 3 (non-pathogenic) – SB1

Gallid alphaherpesvirus 2 (= Marek's Disease Virus)

Meq protein has a key role in oncogenicity, is involved in neurovirulence, is relevant for virulence among others: proline rich repeats (PRRs; 4-proline stretches)

The more proline repeats the lower the virulence

Marek's Disease

Screening PCR

DIVA PCR (CVI988 – RN1250 possible with CVI988 detection system)

Sequencing of meq gene for number of proline repeats

HVT recombinant vaccine strains: HVT-based PCRs (HVT screening or DIVA PCRs based on inserts or overlapping genetic sequence 'unique fingerprint of the construct')

Possible qPCR with proper quantification at day 21 – Gimeno's Technique

Newcastle Disease

Patho-genotyping?

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RESEARCH ARTICLE

Open Access

Observation of risk factors, clinical manifestations and genetic characterization of recent Newcastle Disease Virus outbreak in West Malaysia



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Background: Newcastle disease virus remains a constant threat in commercial poultry farms despite intensive vaccination programs. Outbreaks attributed to ND can escalate and spread across farms and states contributing to major economic loss in poultry farms.

Results: Phylogenetic analysis in our study showed that eleven of the samples belonged to genotype VIIId. All farms were concurrently positive with two immunosuppressive viruses; Infectious Bursal Disease Virus (IBDV) and Marek's Disease Virus (MDV). Amino acid sequence analysis confirmed that eleven of the samples had sequence motifs for velogenic/mesogenic strains; three were lentogenic.

Conclusion: In conclusion, no new NDV genotype was isolated from the 2011 NDV outbreak. This study suggests that the presence of other immunosuppressive agents such as IBD and MDV could have contributed to the dysfunction of the immune system of the chickens, causing severe NDV outbreaks in 2011. Risk factors related to biosecurity and farm practices appear to have a significant role in the severity of the disease observed in affected farms.

Newcastle Disease

Patho-genotyping?



Fig. 1 Clinical signs observed from the outbreak. **a** A typical torticollis is shown. These symptoms normally occur 7 to 10 days after a complaint of high mortality is reported. **b** In severely affected birds, mild swollen head and dyspnea with profuse secretions in the trachea were found. **c & d** Hemorrhagic & necrosis of intestines especially the caecal tonsils & peyer's patches were found. **e** Upon PM, the trachea was severely congested and late in the disease stages pericarditis, perihepatitis and caseous air sacculitis were observed. **f** Proventricular hemorrhages were consistent. **g, h & i** Bursa atrophy was also commonly found in the outbreak. The cut surfaces of the bursa were hemorrhagic – quite atypical from ND infection which prompted us to look for other infectious agents. Not shown above was atrophic thymus.

Table 7 The F cleavage site and it's pathotypes from the Malaysian isolates

Isolate	Genbank* accession no	F cleavage site	Genotype	Pathotype	Source
F1	JN613112	RRRKRF	VIIId	Velogenic/Mesogenic	This study
F2	JN613113	RRRKRF	VIIId	Velogenic/Mesogenic	This study
F3	JN613114	RRRKRF	VIIId	Velogenic/Mesogenic	This study
F4	JN613115	RRRKRF	VIIId	Velogenic/Mesogenic	This study
F5	JN613116	GRQGRL	II	Lentogenic	This study
F6	JN613117	GRQGRL	II	Lentogenic	This study
F7	JN613118	GKQGRL	I	Lentogenic	This study
F8	JN613119	RRRKRF	VIIId	Velogenic/Mesogenic	This study
F9	JN613120	RRRKRF	VIIId	Velogenic/Mesogenic	This study
F10	JN613121	RRRKRF	VIIId	Velogenic/Mesogenic	This study
F11	JN613122	RRRKRF	VIIId	Velogenic/Mesogenic	This study
F12	JN613123	RRRKRF	VIIId	Velogenic/Mesogenic	This study
F13	JN613124	RRRKRF	VIIId	Velogenic/Mesogenic	This study
F14	JN613125	RRRKRF	VIIId	Velogenic/Mesogenic	This study

Newcastle Disease

Patho-genotyping?

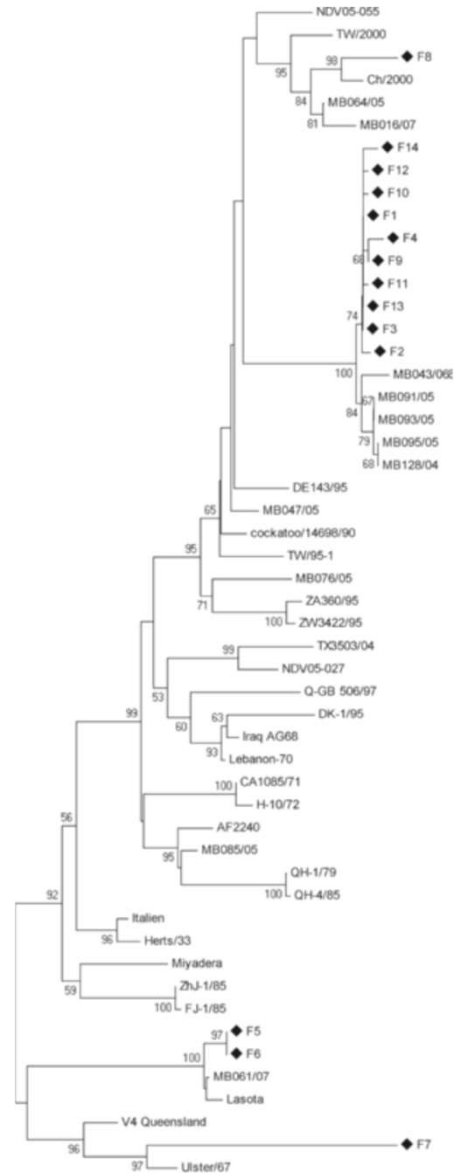
Table 5 Zootechnical results, clinical and necropsy findings

Risk factor	Affected farms	Negative controls
Age of first occurrence of disease	15.9 ^a	28.0 ^a
Age of clinical and necropsy examination	24.8 ^a	32.5 ^a
Mortality at grow-out, %	32.3 % ^a	4.5 % ^b
Presence of respiratory disease	100 % ^a	100 % ^a
Presence of enteric disease	100 % ^a	0 % ^b
Torticollis and neurological signs	91.7 % ^a	0 % ^b
Haemorrhages in more than 1 visceral organ	83.3 % ^a	0 % ^b
Thymus atrophy	100 % ^a	0 % ^b
Bursal atrophy	75 % ^a	50 % ^b
Ascites	8.3 % ^a	50 % ^a
Air-sacculitis, perihepatitis and peritonitis	16.7 % ^a	50 % ^b
Clouded air sacs	50 % ^c	100 % ^d

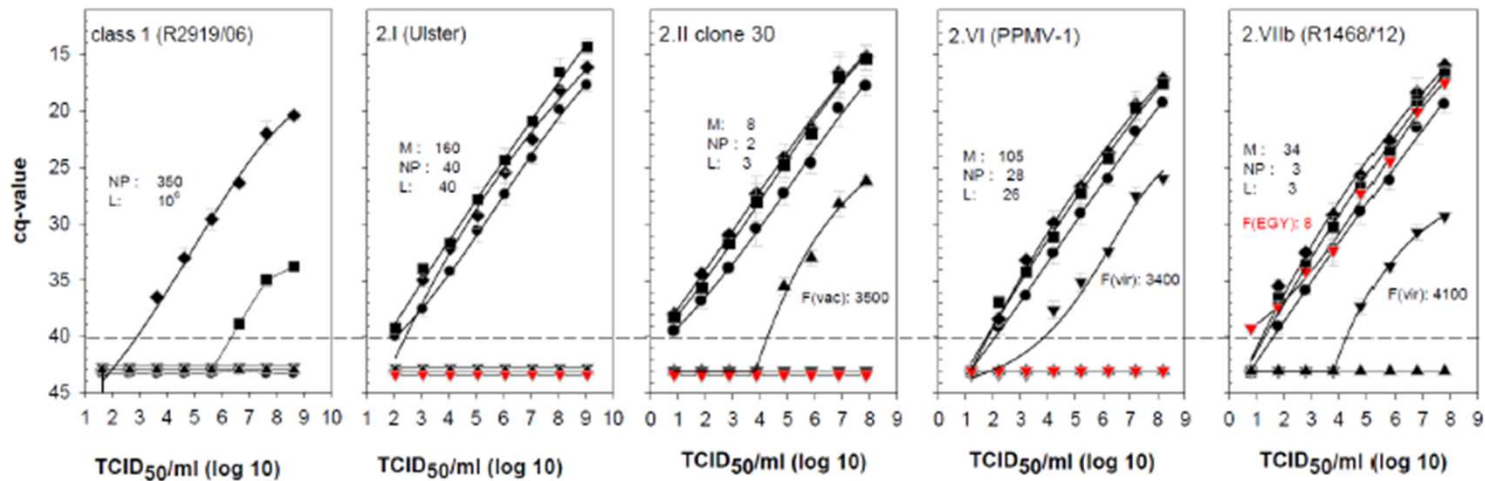
Note: ^{a,b}Values in different columns bearing different superscripts are significantly different ($P < 0.05$), ^{c,d}Values in different columns bearing different superscripts are significantly different ($P < 0.01$)

Table 6 Detection of infectious agents concurrently with NDV positive samples

Presence of concurrent infectious agent in disease farms	Frequency of detection
IBD	83.0 %
MD	83.0 %
IBD+MD	75.0 %
MD Serotype 1	58.0 %
MD Serotype 2	75.0 %
MD Serotype 3	67.0 %



Newcastle Disease



- Limited sensitivity of pathotype specific RT-qPCR
- Adapted reagents close gap in sensitivity between generic and pathotype specific F-RT-qPCR

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Special case of WOAH list A Avian Influenza – HPAI H5Nx

Example of surveillance program (EU – France – Vaccinated Duck Population)

The objective of surveillance is to **ensure that there's no viral circulation amongst the vaccinated flocks**, This is to be made in all epidemiological units (one epidemiological unit is one farm)

1/ **Event based surveillance** is to ensure earliest detection of viral circulation, **any abnormal behavior or clinical signs** are to be declared without delay to veterinarian.

2/**Enhanced passive surveillance**: The sampling protocol involves the taking by the farmer or a technical worker of **tracheal or oropharyngeal swabs** from recently deceased birds up to a maximum of **five dead birds per week** to perform RT-PCR.

3/**Active surveillance**: objective is to detect low level circulation, to be **made by an official vet**, is based on a **clinical examination**, with the **evaluation of the zoo-technical criteria**, completed with a **monthly virological surveillance**. samples are taken from **60 vaccinated birds for PCR and 20 samples for NP ELISA serology**.

HPAI post-vaccination enhanced surveillance and the serological survey		
Parameters	Enhanced passive surveillance	Active surveillance
Where?	The epidemiological unit	
Who?	Farmer or technical worker	Official veterinary
Frequency?	Weekly	Every 30 days: virological testing On batch completion: serological testing
How?	Swabs (tracheal/oropharyngeal) from 5 dead birds	Every 30 days : Swabs (tracheal/oropharyngeal) from 60 birds; At batch completion: blood samples from 20 birds
Testing?	Virological using M gene RT-PCR (If the result is positive, screening for H5/H7)	Virological using M gene RT-PCR (If the result is positive, screening for H5/H7) and NP ELISA serology
Type of laboratory?	A recognised laboratory	An approved laboratory

Thank you for your Attention!