

IBD status update

Q3 2023

Willem Dekkers

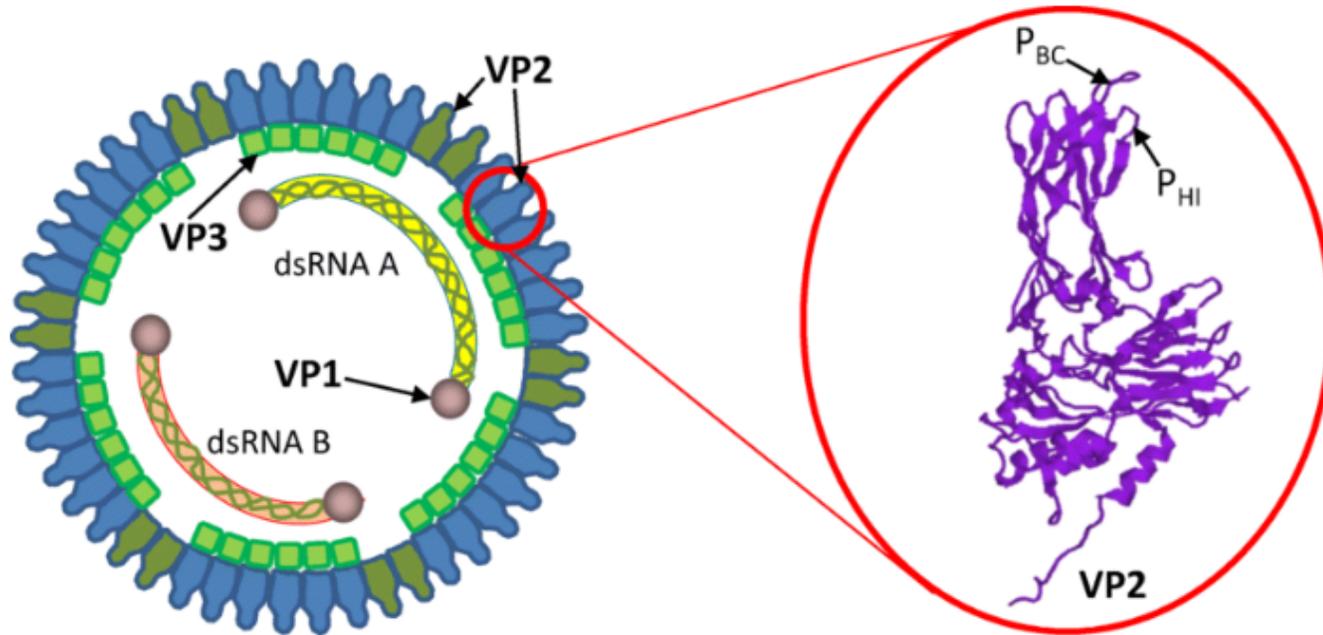


Gumboro-virus (IBDV)



- **Avibirna-virus: 2 segments of dsRNA**
 - Segment A encodes **VP2**, **VP3**, VP4 (ns) and VP5 (ns)
 - Segment B encodes **VP1**: virus polymerase
- **No envelope: very resistant**
 - 52 days survival in water, feed and manure
 - 122 days after removal infected flock still present in the chicken house
 - Sensitive to formaldehyde, chloramine, quats

Main proteins



- **VP2:** outside capsid, neutralizing epitopes, part pathogenicity
- **VP3:** inside capsid
- **VP1:** polymerase, part pathogenicity

Schematic representation of the IBDV structure. The IBDV genome consists of a bipartite double-stranded RNA (dsRNA) and the viral capsid (700 Å in diameter) consists of a single shell formed by 260 trimers of VP2 (780 subunits) and VP3 (600 subunits) organized in a T = 13 icosahedral lattice. Few copies of the RNA-dependent RNA polymerase VP1 are present inside the virion. The arrows in the ribbon diagram of the major protective VP2 antigen (right) show the two hydrophilic regions at the outmost part of the P domain (P_{BC} and P_{HI}) that are critical for virus neutralization

Rage, E., Marusic, C., Lico, C., Baschieri, S., & Donini, M. (2020). Current state-of-the-art in the use of plants for the production of recombinant vaccines against infectious bursal disease virus. *Applied microbiology and biotechnology*, 104(6), 2287-2296.

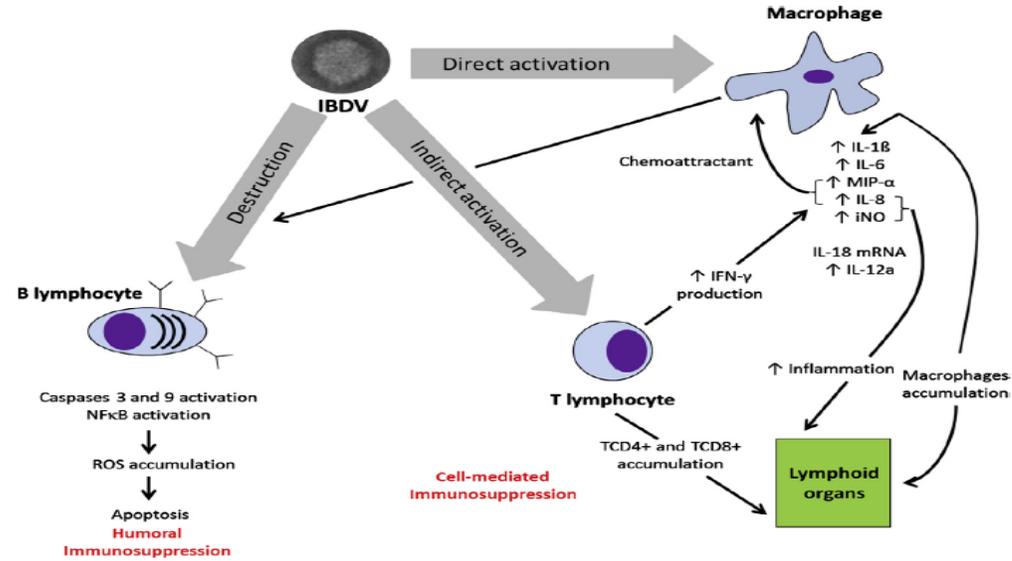


Fig. 2. Interactions between IBDV and the host immune cells (IBDV = infectious bursal disease virus, ROS = reactive oxygen species, iNOS = inducible nitric oxide synthetase, IL = interleukine, IFN = interferon, MIP = macrophage inflammatory protein).

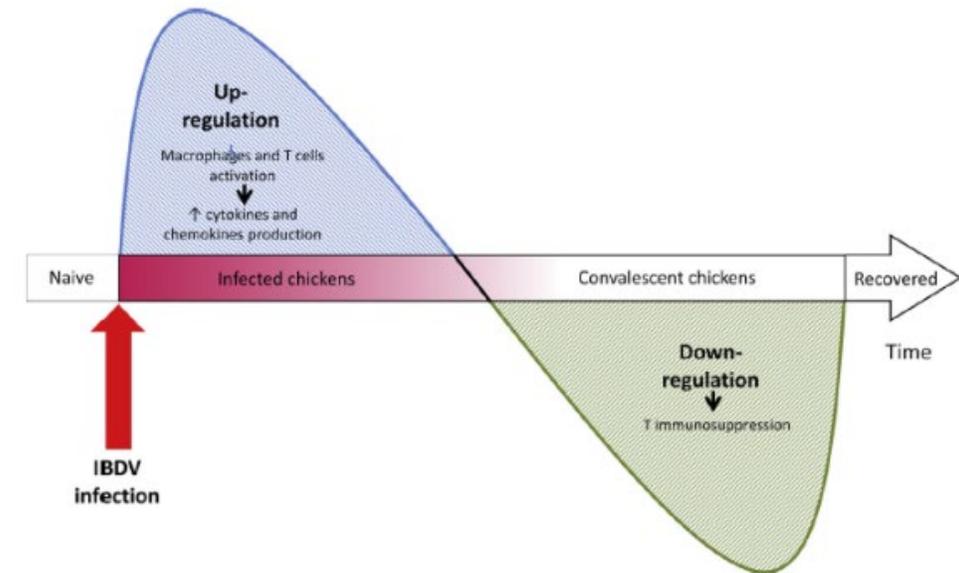
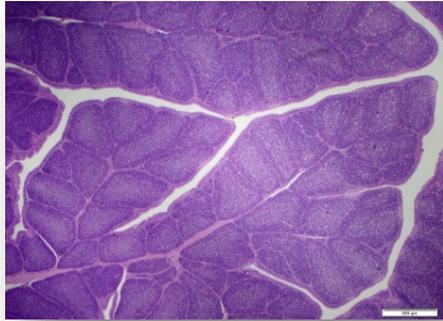


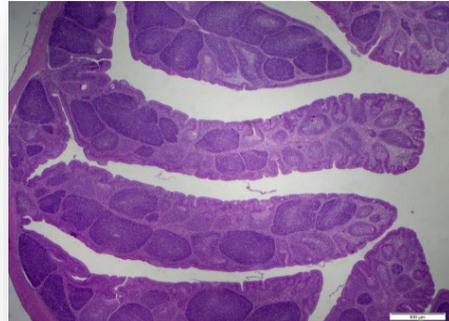
Fig. 3. The pivotal role of ChIFN γ in the immunopathology of IBDV.

Bursa lesion score (Muskett)

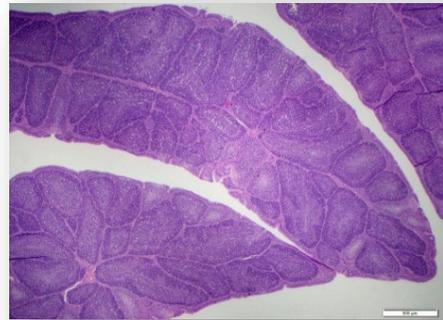
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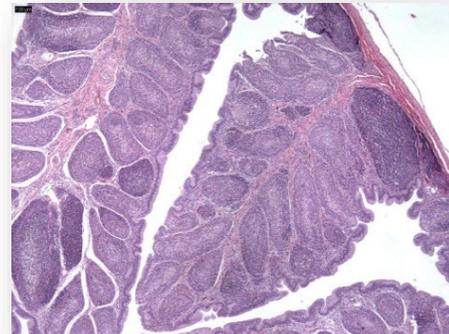
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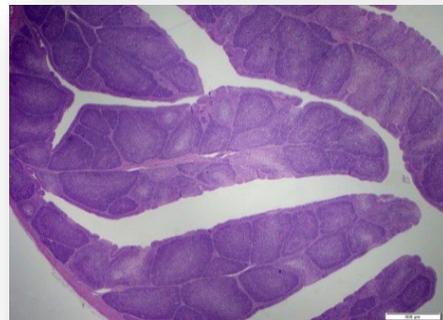
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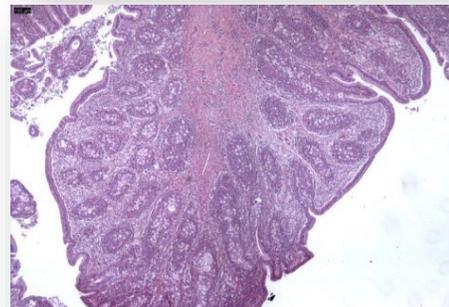
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2



5



Score	Description
0	No lesions, normal bursa.
1	1 percent to 25 percent of the follicles show lymphoid depletion (i.e. less than 50 per cent depletion in one affected follicle), influx of heterophils in lesions
2	26 percent to 50 percent of the follicles show nearly complete lymphoid depletion (i.e. more than 75 per cent depletion in one affected follicle), affected follicles show necrosis and severe influx of heterophils may be detected.
3	51 per cent to 75 percent of the follicles show lymphoid depletion; affected follicles show necrosis and severe influx of heterophils may be detected.
4	76 percent to 100 percent of the follicles show nearly complete lymphoid depletion, hyperplasia and cyst structures are detected; affected follicles show necrosis and severe influx of heterophils is detected.
5	100 percent of the follicles show nearly complete lymphoid depletion; complete loss of follicular structure, thickened and folded epithelium, fibrosis of bursal tissue.

Damage of IBDV infections



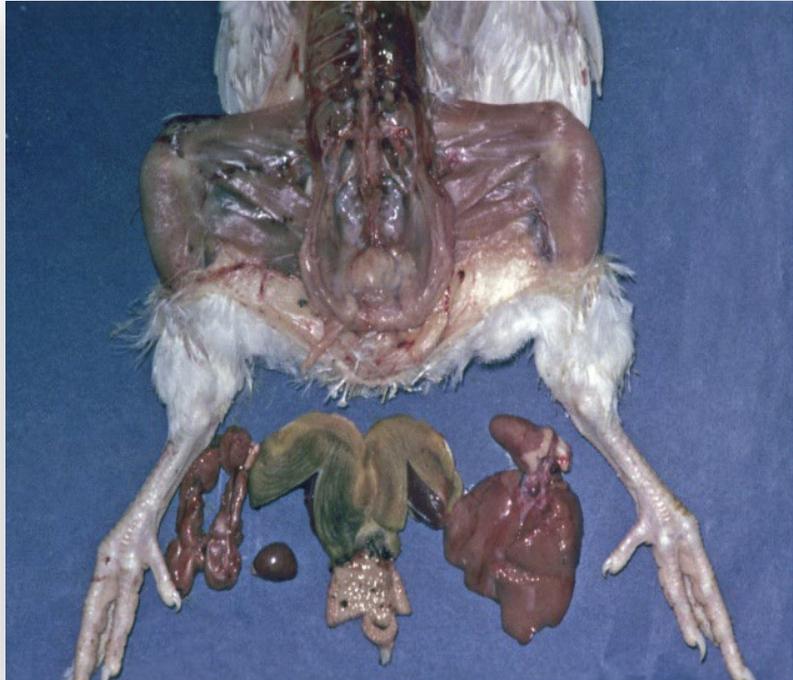
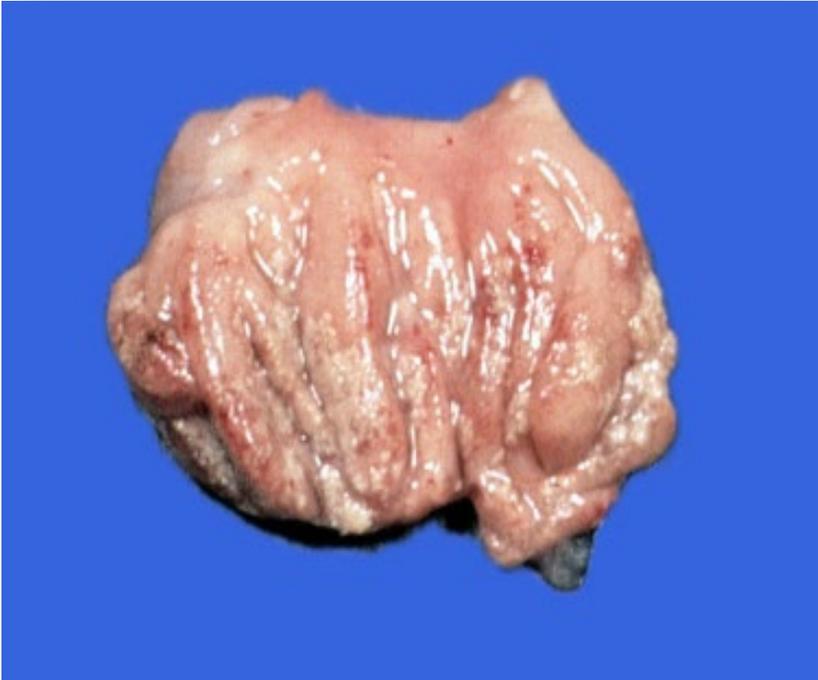
- Age dependent
- Kind of bird (broiler/layer)
- Strain dependent:
 - mortality between 0 and 100%, immune-suppression*
- MDA dependent (very early challenge)
- Immune-suppression:
 - damage depends on other challenges*
- Vaccinated?



Clinical signs



- short incubation period: 3 - 4 days
- In sensitive young birds:
 - acute mortality
 - in 2 - 3 days reaching a peak value
 - after the peak a rapid decline in mortality



Symptoms subclinical IBD



- growth depression
- poor feed conversion (FCR)
- sometimes some diarrhoea
- problems due to immunosuppression
- more reaction to vaccinations, lower response to vaccinations
- difficult to diagnose (clinic, serology, histopathology)



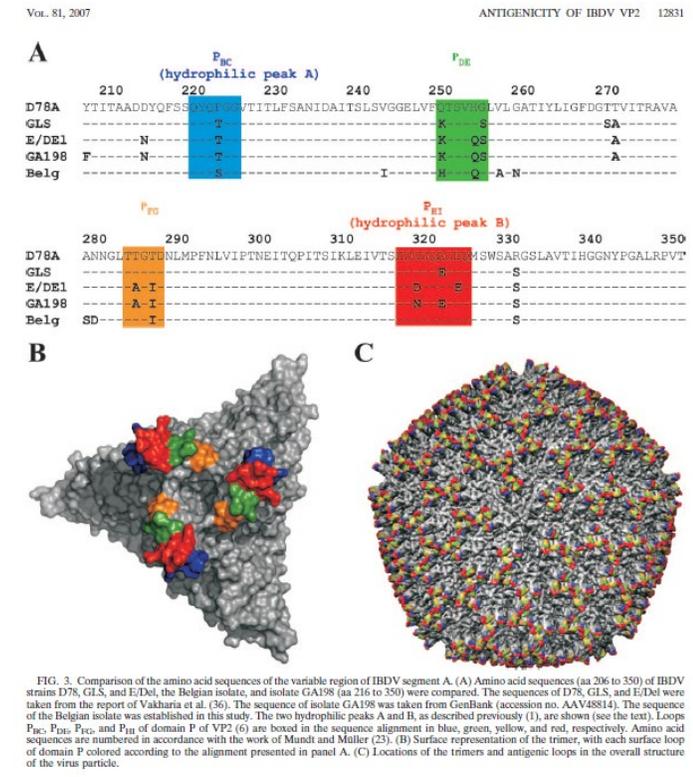
IBDV strain typing



- **Main proteins IBDV:**

- **VP2:** outside capsid, neutralizing epitopes, part pathogenicity
- **VP3:** inside capsid
- **VP1:** polymerase, part pathogenicity

- Pathotype: VP2 and VP1
- Protection: passive immunity (MDA), active immunity (live vaccine)
- Serotype (VNT): VP2
- Epitope type (Moab, IFA, ELISA): VP2
- Genotype (RT-PCR, RFLP, sequencing): VP2, best also VP1



Letzel et al, 2007, Journal of Virology

Serotype 1:

- **Classical-like stains (Faragher, vvIBDV, vaccines)**
 - Clinical signs, swelling, atrophy of bursa
 - Illness and immunosuppression
 - Main strains Africa, Asia, Europe, Latin America
- **Variant-like strains (Del E, GLS, etc.)**
 - No clinical signs, atrophy of bursa
 - Immunosuppression
 - Main strains in USA, also detected in other parts of the world

Serotype 2:

- turkeys, no clinical symptoms in the chicken



Apathogenic
(serotype 2, HVT-VP2)



No mortality, no bursal lesions

Pathogenic

- Mild
- Intermediate
- Intermediate plus
- Hot
- Classical
- Variant
- Very virulent



No mortality, some bursa-lesions



More bursal lesions



High mortality + severe lesions

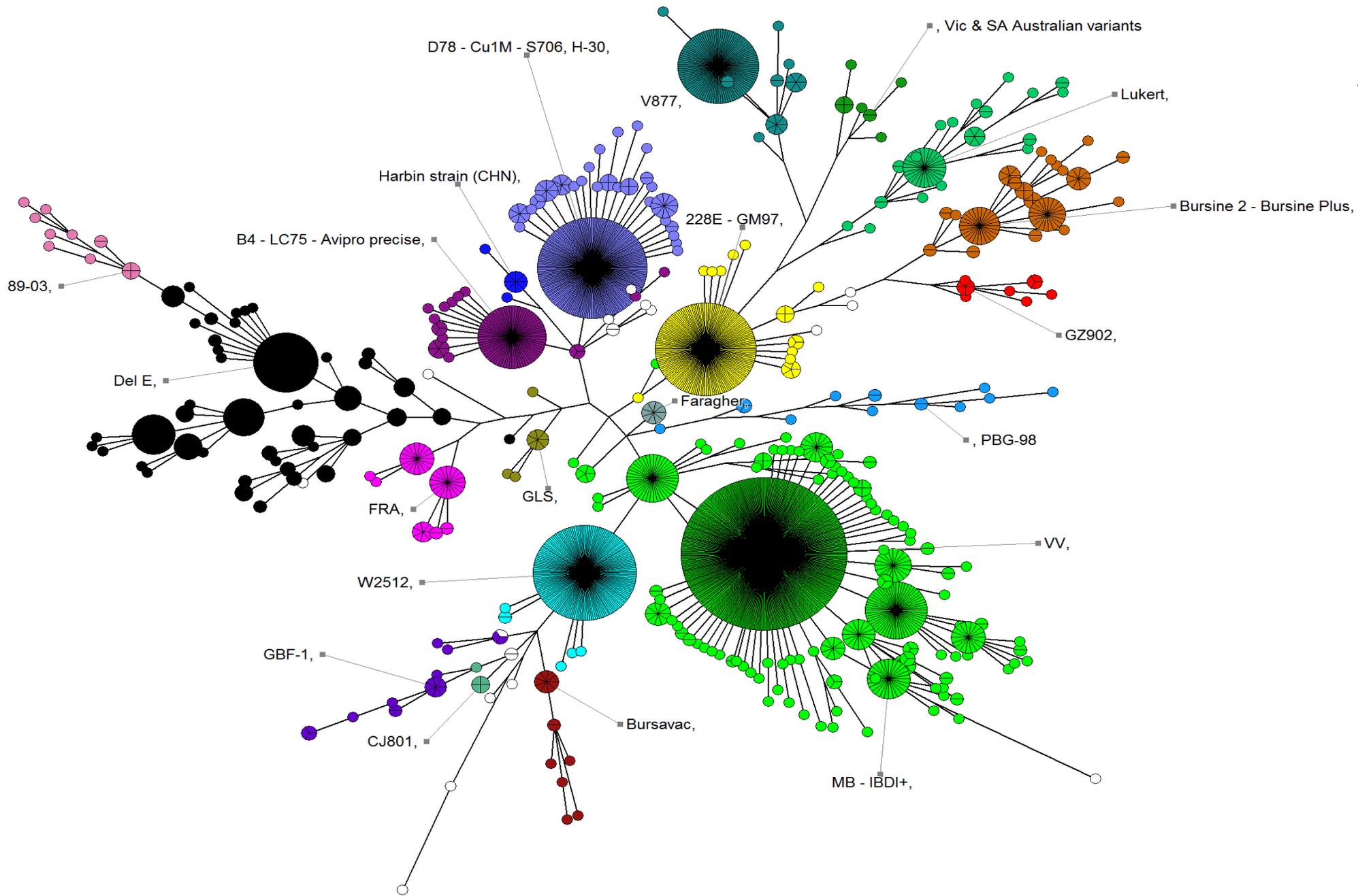
Differences between LAV and field strains

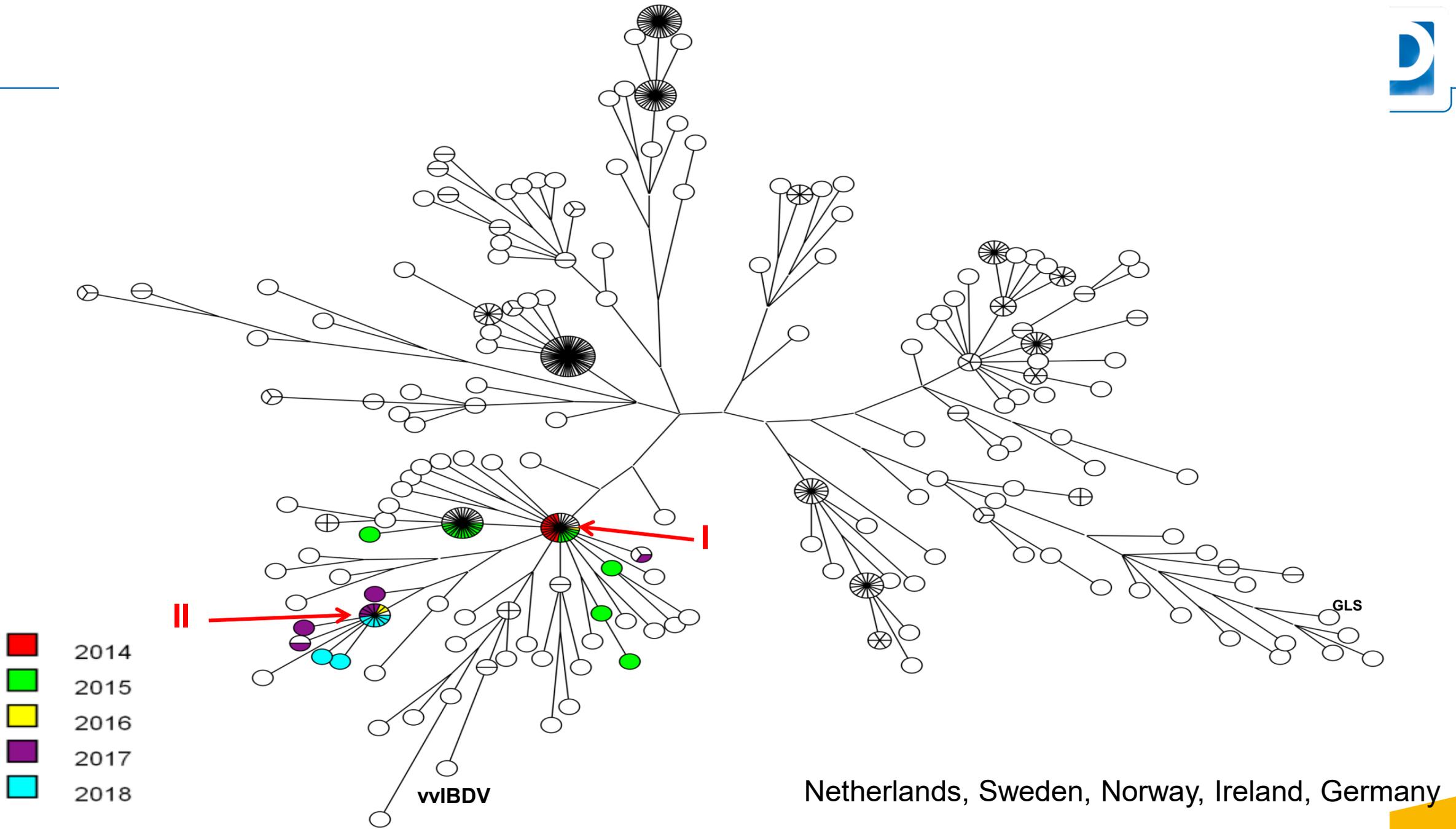


Pathotype	Clinical signs, mortality	Bursal lesions	Immune-suppression	Breakthrough (classical) MDA
Non-pathogenic	No	Hardly	No	Very low
<i>HVT-VP2</i>	<i>No</i>	<i>No</i>	<i>No</i>	<i>Not applicable</i>
Mild	No	Some	No	Very low
Intermediate	No	Some more	No(hardly)	Low (e.g.125)
Intermediate plus	Mild signs in some birds, no mortality	More and longer	No relevant level (field)	Higher (e.g. 500)
Hot	Mild signs in more birds, no mortality	More and slow recovery	Might be relevant	High (e.g. 1000-2000)
Classical	Low till 10% mortality		Relevant	High (e.g. 1000-2000)
Biological variant	Low, hardly mortality	severe	Very relevant	High
vvIBDV	High mortality	severe	Very relevant	High (around 2000)

Pathotypes (general scheme!!, strain, age, MDA, etc. dependent)

- Product of RT-PCR is analyzed for the sequence of all nucleotides.
- The sequence of nucleotides can be translated into the sequence of amino acids (AAs)
- Comparison with known sequences of other strain
 - **Percentage of similarity**
 - **Phylogenic tree**





Research for the Dutch poultry sector 2019



- Pathogenicity study
- SPF layers: ruffled feathers 3-5 dpi
- SPF broilers: no clinical signs
- Non recovering
- Severe bursa lesions

Tabel 3. Gemiddelde bursa-/lichaamsgewicht-verhoudingen (x1.000) (BLV) en gemiddelde bursalaesiescores (BLS) per groep, 10 en 21 dagen na de besmetting

GROEP	DIER-TYPE	CHALLENGE	MORTALITEIT POST CHALLENGE	STUDIE-DAG	AANTAL DIEREN	GEMIDDELDE BLV	GEMIDDELDE BLS
		D14	[D14-D35]				
1	SPF-opfokleg	98,1% DV88	0%	D24 (10 dpc)	10	1,00	4,2
				D35 (21 dpc)	10	0,89	4,1
2	SPF-vleeskuiken	98,1% DV88	0%	D24 (10 dpc)	10	0,87	4,5
				D35 (21 dpc)	10	0,35	4,5
3	SPF-opfokleg	Steriel water	0%	D24 (10 dpc)	2	7,88	0,0
				D35 (21 dpc)	3	5,73	0,0
4	SPF-vleeskuiken	Steriel water	0%	D24 (10 dpc)	2	2,21	0,0
				D35 (21 dpc)	1	4,12	0,0

GROEP	DIER-TYPE	CHALLENGE	STUDIE-DAG	AANTAL DIEREN	AANTAL DIEREN PER BURSALAESIESCORE					
					0	1	2	3	4	5
1	SPF-opfokleg	98,1% DV88	D24 (10 dpc)	10	0	0	0	0	8	2
			D35 (21 dpc)	10	0	0	0	0	9	1
2	SPF-vleeskuiken	98,1% DV88	D24 (10 dpc)	10	0	0	0	0	5	5
			D35 (21 dpc)	10	0	0	0	0	5	5
3	SPF-opfokleg	Steriel water	D24 (10 dpc)	2	2	0	0	0	0	0
			D35 (21 dpc)	3	3	0	0	0	0	0
4	SPF-vleeskuiken	Steriel water	D24 (10 dpc)	2	2	0	0	0	0	0
			D35 (21 dpc)	1	1	0	0	0	0	0

- Breakthrough titer (MDA's)
- Higher than 1.000 Idexx ELISA
- (n=1: >1.266 for the 98,1% DV86)

Tabel 5. Aantal dieren per bursalaesiescore op 4 dagen na besmetting met Gumboro

GROEP	IBDV-ELISA GEMIDDELDE TITER (D4)	GUMBORO- CHALLENGE- VIRUS (D4)	D8						
			AANTAL DIEREN PER BURSALAESIESCORE						
			n	0	1	2	3	4	5
1	122	D6948	8					3	5
2	227	D6948	8				1	7	
3	375	D6948	9				3	3	3
4	375	D6948	4		1			3	
5	541	D6948	4			1		2	1
6	40	98,1% DV86	8					1	7
7	285	98,1% DV86	8				1	6	1
8	243	98,1% DV86	9				1	8	
9	417	98,1% DV86	3				2	1	
10	940	98,1% DV86	4					3	1



Occurrence and spread of a reassortant very virulent genotype of infectious bursal disease virus with altered VP2 amino acid profile and pathogenicity in some European countries

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ABSTRACT

Reassortant strains of Infectious Bursal Disease Virus (IBDV) were detected in commercial broiler flocks in the Netherlands, Belgium, Denmark, Czech Republic and Germany and in layers and organic broilers in Sweden in the period of 2017–19. Genetic analysis, based on hypervariable region of VP2 gene showed grouping together with very virulent IBDV strains (vvIBDV, Genogroup 3), but these recent viruses formed a separate cluster, which was most closely related to Latvian IBDV strains from 2010–13. VP1 gene of these isolates was most closely related to D78 attenuated IBDV strain. The recently described reassortant IBDV strain (Bpop/03/PL) from Poland with similar genomic constellation (segment A from vvIBDV, segment B from attenuated strain) retained its pathogenicity (80 % mortality in SPF chickens). Infection with the North-West European reassortant IBDVs described in this study showed subclinical feature in the field (without complicating agents) and when tested under standardized pathogenicity test in SPF layer chickens (no mortality or clinical signs, but marked bursa atrophy was observed). Although these recent North-West European reassortant strains had all amino acid residues in their VP2 gene which are considered as markers of vvIBDV strains, they exhibited typical amino acid changes compared to vvIBDV reference strains that should contribute to the determination of pathogenicity. Diagnostic investigations indicated that co-infection with fowl adenovirus or chicken infectious anaemia virus exaggerated the outcome of the IBDV infection (10–20 % mortality). Widespread presence of this reassortant IBDV group in clinically healthy flocks draws attention to the importance of active surveillance.

Relation VP2 genotyping and pathotyping



- VP2 (A segment of dsRNA): major contribution to pathogenicity
- VP1 (B segment of dsRNA): part of pathogenicity (speed and efficacy of replication)
- Within one strain: VP2 and VP1 of same source (e.g. vvIBDV)
- However: recombinations can occur:
 - VP2 of strain A and VP1 of strain B

Reassortment of segments A and B of IBDV



	Strain	Segment A (VP2)	Segment B (VP1)	pathogenicity
Le Nouen <i>et al</i> , JGV 2006, 209-216	02015 (Venezuela)	vvIBDV	Non-vvIBDV	8% mortality (52% for vvIBDV)
Jackwood <i>et al</i> , Virology, 2011, 98-105	CA-K785 (USA)	vvIBDV	Serotype 2 (USA)	20% mortality (100% for vvIBDV)
Jackwood <i>et al</i> , Avian Diseases, 2016, 765-772	D6337 (USA)	classical	vvIBDV	As cv STC
Soubies <i>et al</i> , Avian Pathology, 2017-19-27	100056 (France)	vvIBDV	Serotype 2 (EU)	Severe bursal atrophy, no mortality
Pikula <i>et al</i> , Vet. Research, 2018, 89	Bpop/03 (Poland)	vvIBDV	D78-like attenuated strain	80% mortality (SPF white layers)
Mato <i>et al</i> , Vet. Microbiology, 2020	D3976/1/17DE (Germany)	vvIBDV (several AA changes)	D78-like attenuated strain	No clinical signs, inflammation bursa, partial regeneration of few remaining follicles (0% after vvIBDV)

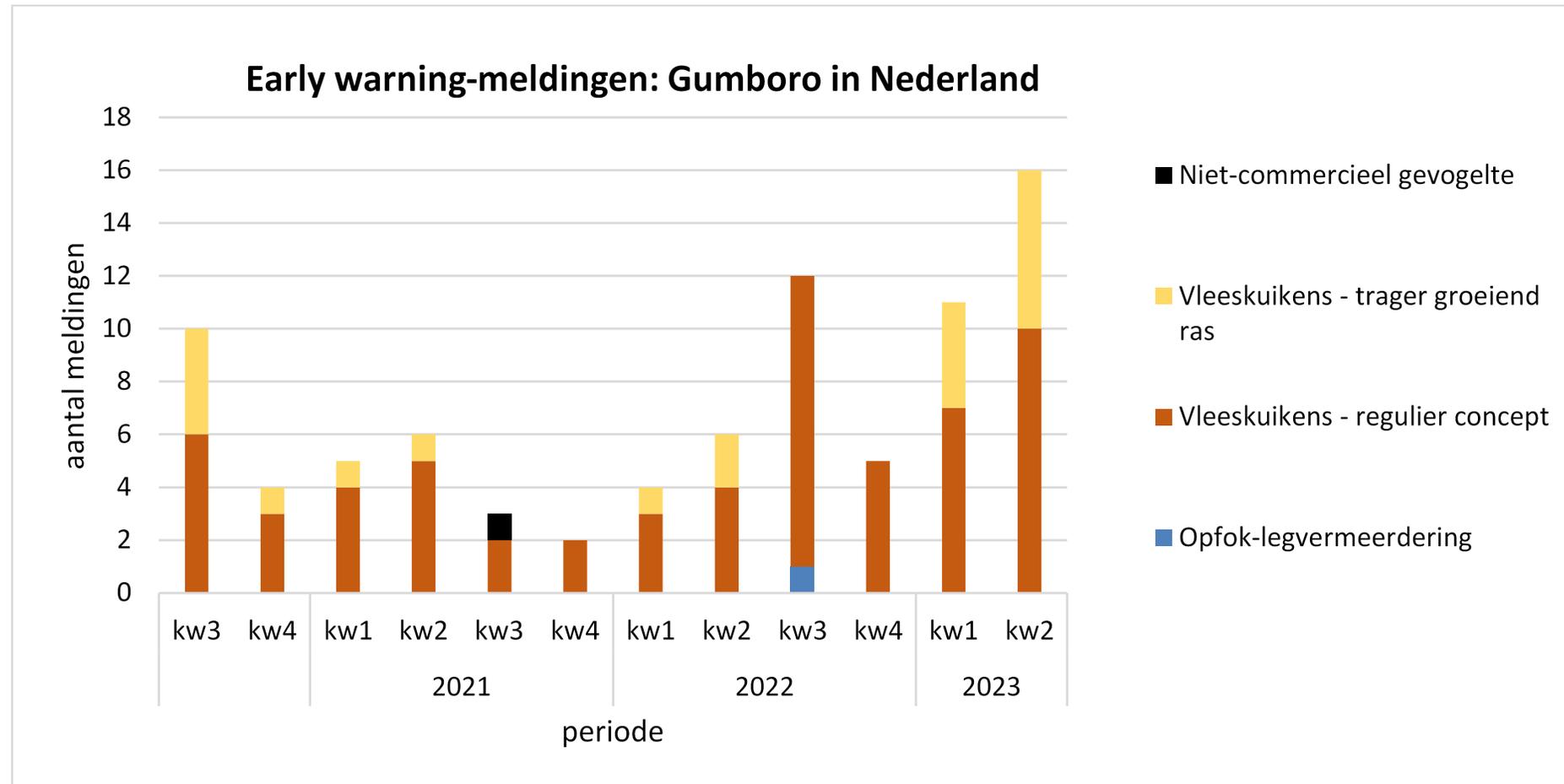
Classification Islam et al 2021



Segment A (VP2, 3, 4, 5)		Segment B (VP1)	
A1	Classical	B1	Classical-like (& Serotype 2-like)
A2	US variant	B2	Very virulent-like
A3	Very virulent	B3	Australian-like
A4	dIBDV	B4	Polish & Tanzanian
A5	Mexican	B5	Nigerian
A6	Italian		
A7	Early Australian		
A8	Australian variant		
A0	Serotype 2		

Early Warning System voor Gumboro

In het eerste halfjaar van 2023 werden zestien meldingen gedaan van een Gumboro-uitbraak. Alle meldingen kwamen voort uit of werden bevestigd via PCR-onderzoek bij GD.



Detection of IBDV infections



- Detection of the virus (including typing):
 - Infectious particles (virus isolation)
 - Antigen (staining)
 - Genome (RT-PCR)
- Detection of the antibody response

Virus detection from (suspected) subclinical cases



No clinical signs, so when to sample?

- *Frequent sampling of 5 bursa's at 2, 3, 4 and 5 weeks (-20°C).*
- *When (retrospective) suspicion of infection (based on performance, serology or other reason):*
testing of bursa material of the different weeks (1 pool per week) for presence of IBDV including typing.

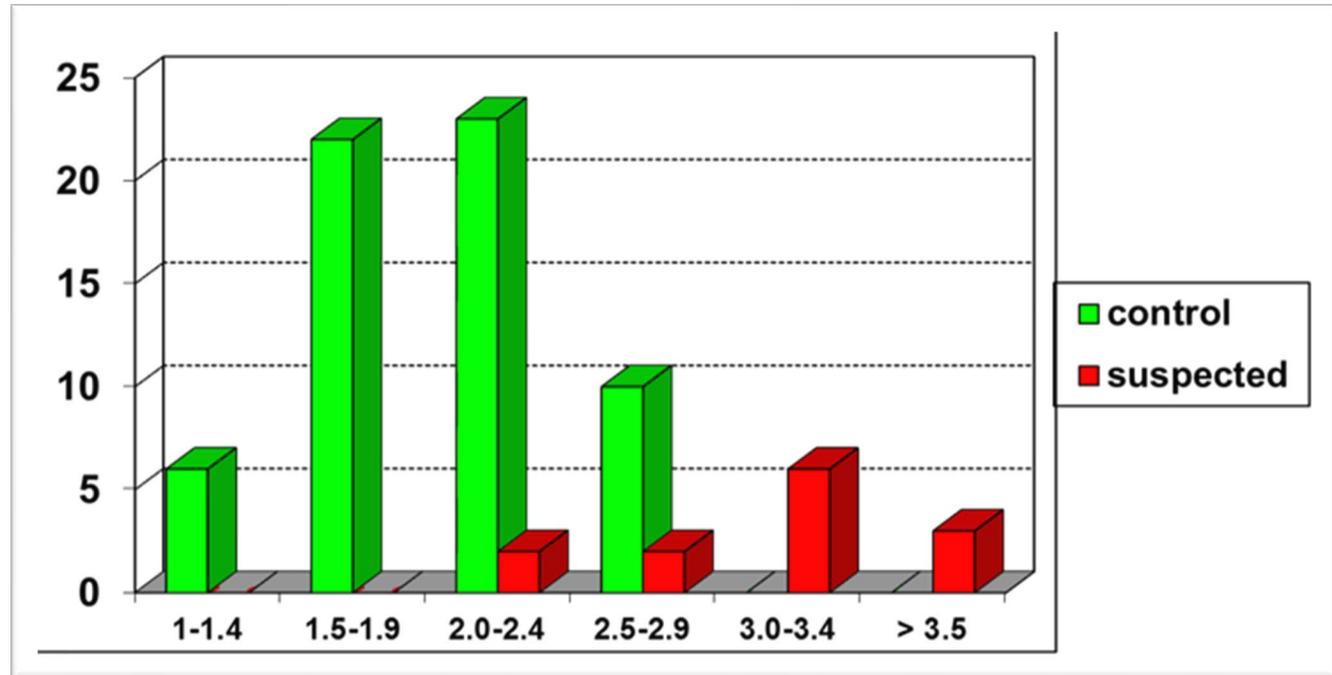
Check for (subclinical) infections in unvaccinated and vaccinated chickens



- Vaccinated birds: increased titres at slaughter is an indication (not a proof) for challenge: look for other signs: e.g. feed conversion, wet litter, PM, etc.

- However: no clear cut-off

*ELISA results at 6 weeks
in vaccinated unsuspected broilers flocks
vs.
IBDV-suspected broilers flocks*





Clinical and subclinical infections (do not underestimate them)

- Biosecurity, management
- Cleaning and disinfection
- Passive immunity (maternally derived antibodies)
- Active immunity (vaccination)

- Kind of vaccines (available):
 - Conventional live attenuated vaccines
 - Antigen/antibody complex vaccines
 - Recombinant vaccines
 - Inactivated vaccines (boosting)

Live attenuated vaccines (LAV)



- Drinking water (spray, eye-drop)
- Vaccine strain: mild, intermediate, intermediate plus, hot
- Less attenuated (more virulent):
 - Higher breakthrough titre (earlier vaccination)
 - Increased level of damage to the follicles
 - Longer period of damage to the follicles
- Fast protection after application when MDA is below breakthrough titre (each bird), neutralised when MDA is too high
- Spreads
- Application age depending on level of MDA

Antibody/antigen complex vaccines



- In ovo or in hatchery by injection (equipment)
- Vaccine strain: intermediate plus/hot
 - More severe bursal lesions
- Replication starts when the MDA is low enough (each bird)
- Replication under field conditions after between 2.5 and 4 weeks of age
- Spreads
- Full immune response

Competitive exclusion



- Competitive exclusion or Viral interference: A cell infected by a virus becomes resistant toward a second infection by other strain of the same virus.
- Viral interference of IBDV against pathogenic IBDV was described by Ashraf in 2005: A mild IBDV strain interfered with the replication of a pathogenic IBDV strain.
- The property to reduce the replication of the pathogenic field IBDV is an important benefit of LAV.

1. Ashraf, S, G. Abdel-Alim, M.Q. Al-Natour, and Y.M. Saif (2005). Interference between mild and pathogenic strains of infectious bursal disease virus in chickens. *Avian Dis.* 49(1):99- 103.

2. Whitaker-Dowling, P., and J. S. Younger. (1987) Viral interference dominance of mutant viruses over wild type virus in mixed infections. *Microbiol. Rev.* 51:179–191.

Recombinant HVT-VP2 vaccines



- In ovo or in hatchery by injection
- Replication starts after application (Mareks strain), independently from MDA against IBDV
- IBDV vaccine: part of VP2, other proteins are lacking
- Does not spread (missed is unprotected)
- Can not be combined with other HVT vaccines
- High/complete protection after about 2-4 weeks

Alle (jonge) diertypes ook al worden er bij opfok leg en opfok slachtvermeerdering niet vaak gevallen van Gumboro gemeld.

Drie verschillende 'soorten' koppels:

- Koppels met (uiteenlopende) klinische problemen van 2-6 weken leeftijd die ingestuurd worden naar de sectiezaal van GD (buiten dit VMP project om)
- Zes dieren uit koppels met tegenvallende resultaten en sluimerende problemen van 3-6 weken leeftijd die ingestuurd worden naar GD met bijgevoegd **sectie-inzendformulier** waarbij het mag gaan om vers dode dieren of achterblijvers.
- Zes bursa's of zes swabs van zes bursa's van gezonde (controle) koppels van 4-6 weken leeftijd met bijgevoegd **PCR-inzendformulier** waarbij het mag gaan om monsters van achterblijvers.

Timing of the vaccination(s)



- Hatchery
- In the field
 - Which level of MDA?
 - Which variation in MDA
 - Field pressure (hygiene, manure)
 - Which vaccine
 - One or more vaccinations
 - Housing (ground vs cage)
 - Type of bird (layer, broiler, breeder)

Timing of the vaccination(s)



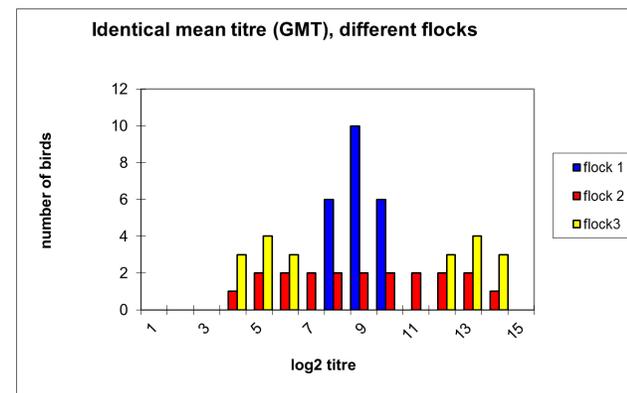
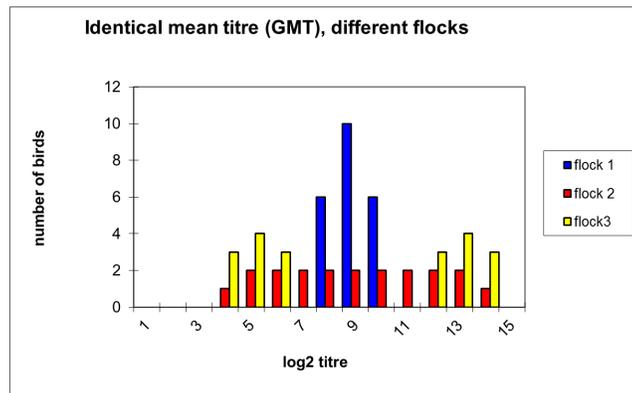
- General rules:
 - low field pressure: age of vaccination based on general information / ideas
 - high field pressure: age of vaccination(s) based on specific information of that flock is better than guessing based on general information
- Need of specific information is depends on the variation of MDA within and between flocks

Estimation of optimal age for Gumboro vaccination



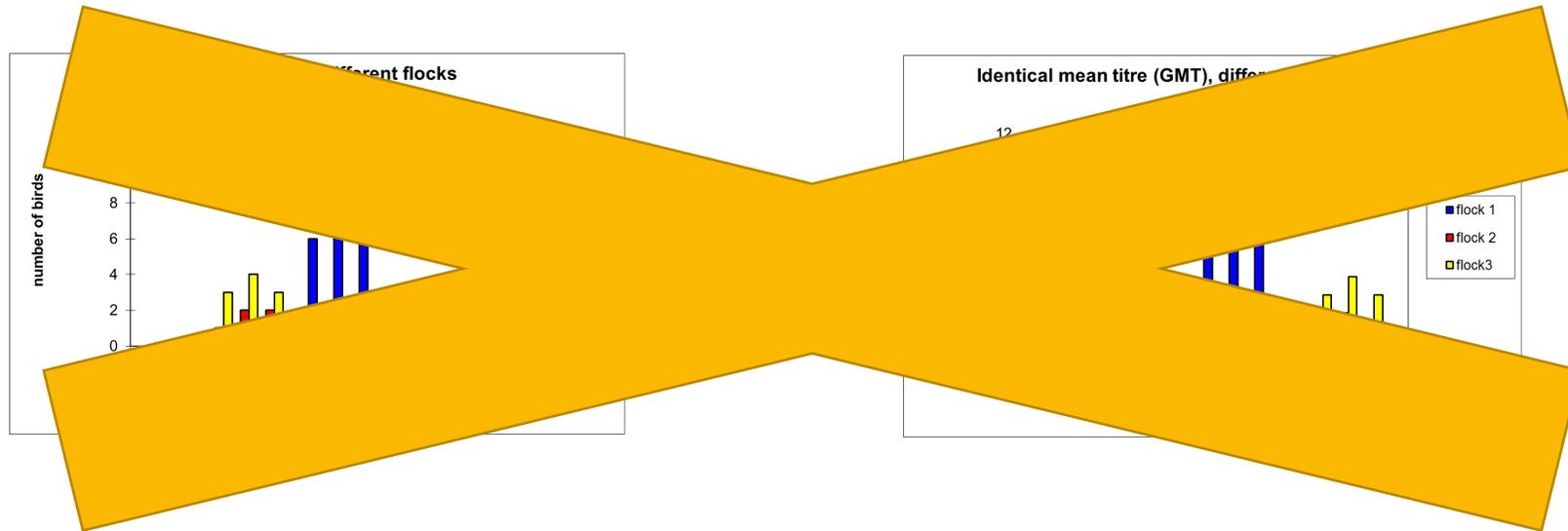
Date estimation:

1. Measure MDA in a good sample of the flock (at least 18 healthy chickens)
2. Calculate when MDA is (almost) gone with a suitable formula



Date estimation:

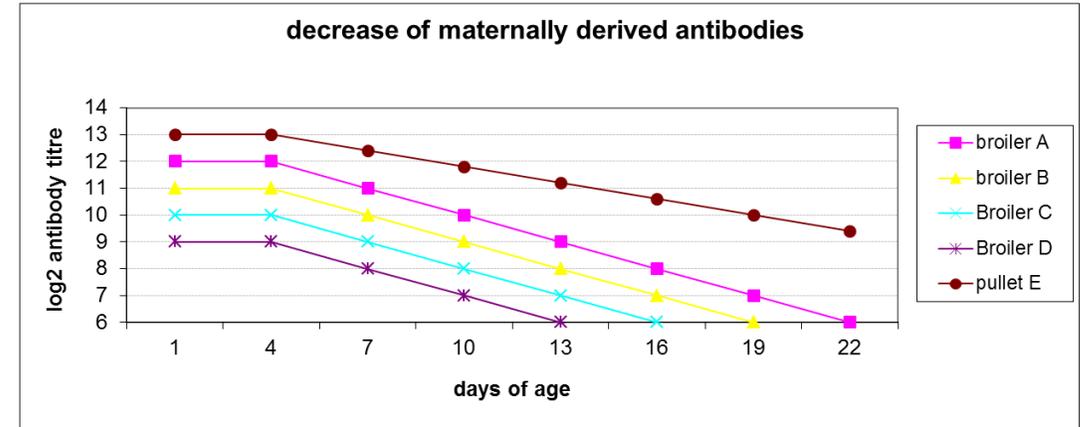
1. Measure MDA in a good sample of the flock (at least 18 healthy chickens)
2. Calculate when MDA is (almost) gone with a suitable formula



Deventer formula



- Vaccinate when a certain percentage of the flock has become susceptible for the vaccine:
 - default % for 1 vaccination: 75%
 - default for 2 vaccinations: 30-40% and 80%
 - adapted for a specific flock when needed:
 - strange flock (variation in titres)
 - special farm, field pressure
 - more vaccinations



- possible variations:
 - $t_{1/2}$: VN = gold standard (3 broiler, 5.5 for layers)
 - % of flock: 75%, 40 and 80% , ? and ?
 - vaccine: mild, intermediate, intermediate plus, hot (different breakthrough titres)
 - number of vaccinations
 - age of sampling

Titers for vaccination date Gumboro vaccination

	Broilers t 1/2 = 3 days	Rearing broiler breeder t 1/2 = 4,5 days	Rearing layer t 1/2 = 5,5 days
Wait for ... days*			
3	256		
4	323	237	212
5	406	276	240
6	512	323	273
7	645	376	309
8	813	439	351
9	1024	512	398
10	1290	597	451
11	1625	697	512
12	2048	813	581
13	2580	948	659
14	3251	1106	747
15	4096	1290	848
16	5161	1505	961
17	6502	1756	1091
18	8192	2048	1237
19	10321	2389	1403
20	13004	2787	1592
21		3251	1805
22		3792	2048
23		4424	2323
24		5161	2635
25		6020	2989
26		7023	3390
27		8192	3846
28		9556	4362
29		11148	4948
30		13004	5613
31		15170	6367
32			7222
33			8192
34			9292
35			10540



Estimation of the optimal time of vaccination



- Only a tool, it is not perfect
- It makes you aware of what is going on (no black box anymore)
- Can not compensate for bad hygiene, mistakes in vaccine application, low dosage of vaccine, etc, etc
 - Different ELISAs (can) have different breakthrough titres and half live times for MDA. Check, ask!
 - Please stick to the original formula (with it's variables), or use another name.

Main causes of “vaccination failures”



- Early, high vvIBDV field pressure
- Early, high variant IBDV pressure (MDA too low, not sufficiently protective)
- Bad timing (mostly too early) of the vaccination(s)
- Application of the vaccine (in the farm or in-ovo)

Thank you for your attention!

