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Using Phylogenetic Analysis to Examine the Changing Strains of Infectious Bronchitis Virus Infections in Ontario Over Time

CAHLN June 6, 2017

Emily Martin, Marina Brash, Margaret Stalker and Davor Ojkić

Animal Health Laboratory

University of Guelph

Guelph, Ontario, Canada

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Outline

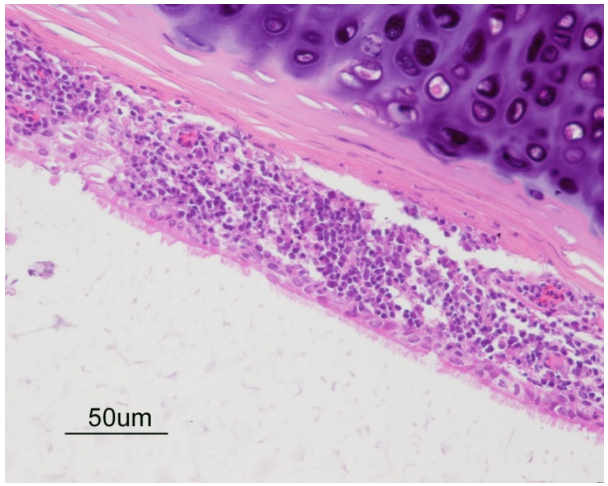
- Virus Characteristics
- IBV in Ontario
  - 2000 to 2012/2013
  - 2012 to 2017
- Clinical signs

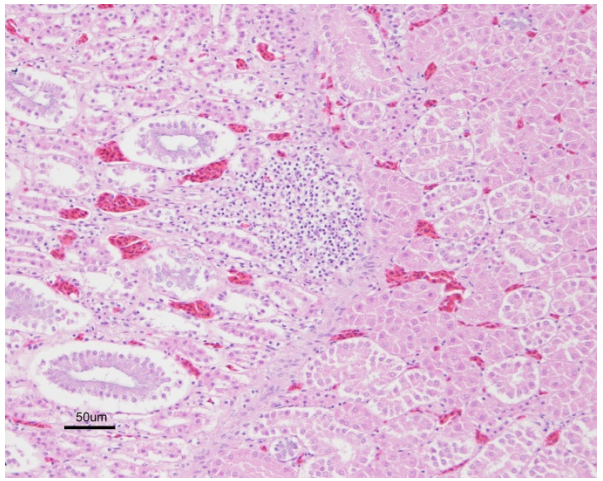
Infectious Bronchitis Virus

- gammacoronavirus, ssRNA, enveloped
- Large surface proteins – spike (S) proteins
  - Role in virus attachment and fusion with host cell
  - Two subunits
    - S1 – neutralizing antibody epitopes
    - S2

IBV - Disease

- Respiratory
- Nephropathogenic
- Reproductive organs
- Egg production problems
- Proventriculitis



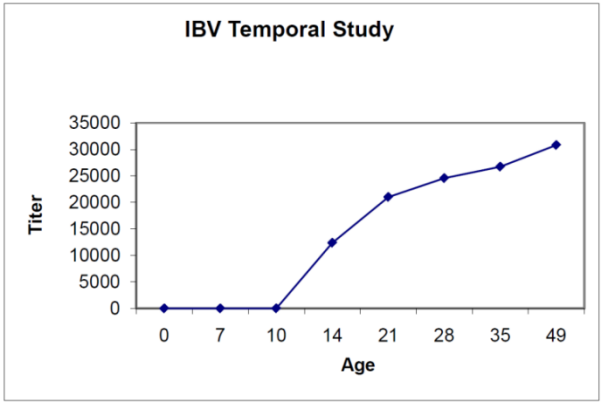


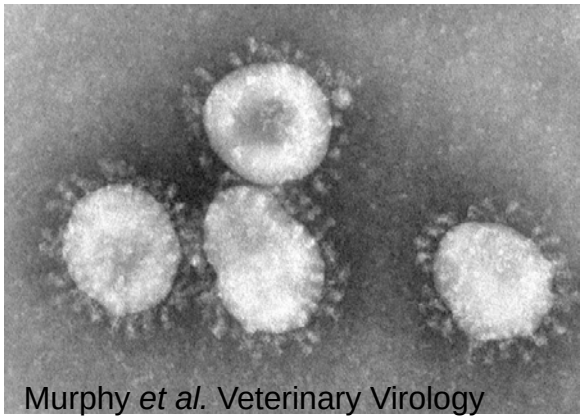
Diagnostics

- Serology
- Virus isolation/EM
- PCR
- Genotyping: S1 gene sequencing

Data

Age	Titer
0	0
7	0
10	12
14	12373
21	21014
28	24579
35	26731
49	30805





Murphy *et al.* Veterinary Virology

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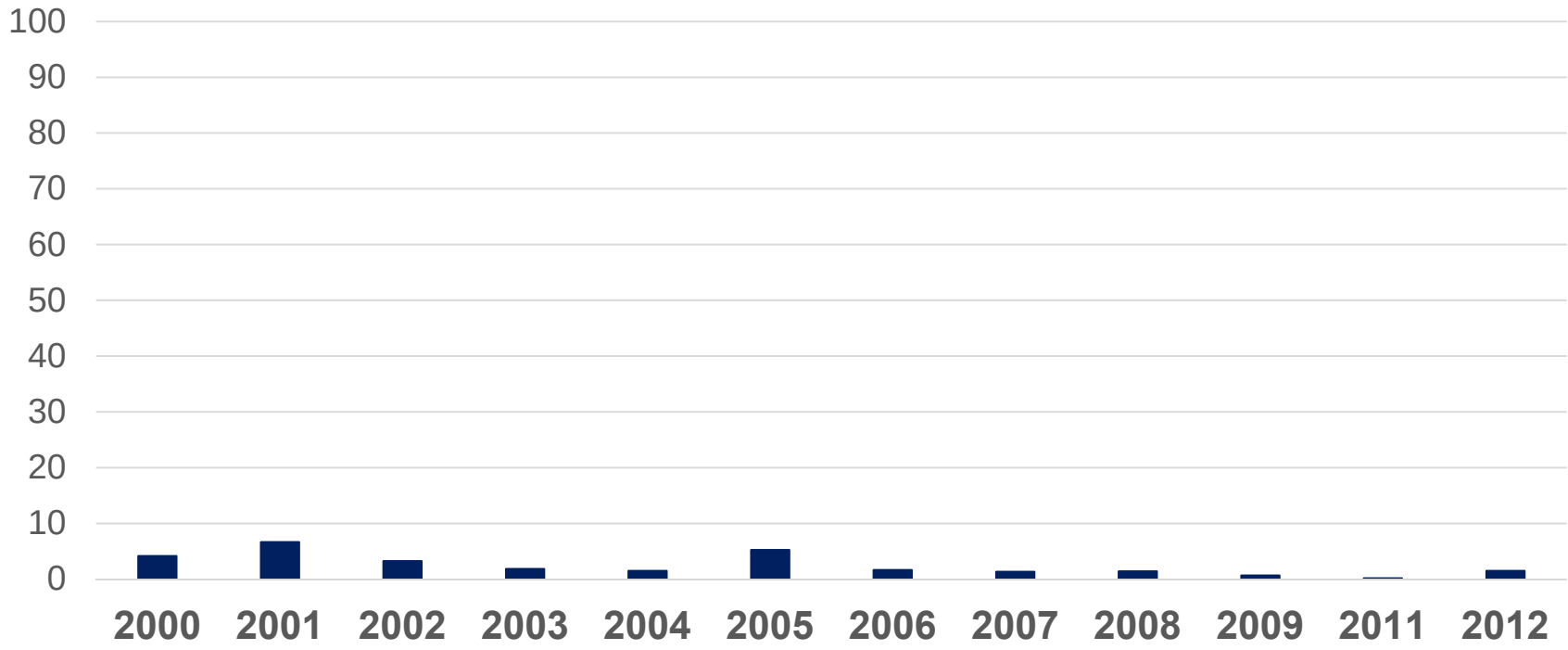
IBV VIRUS ISOLATION (per month, 2000-2012), n=344



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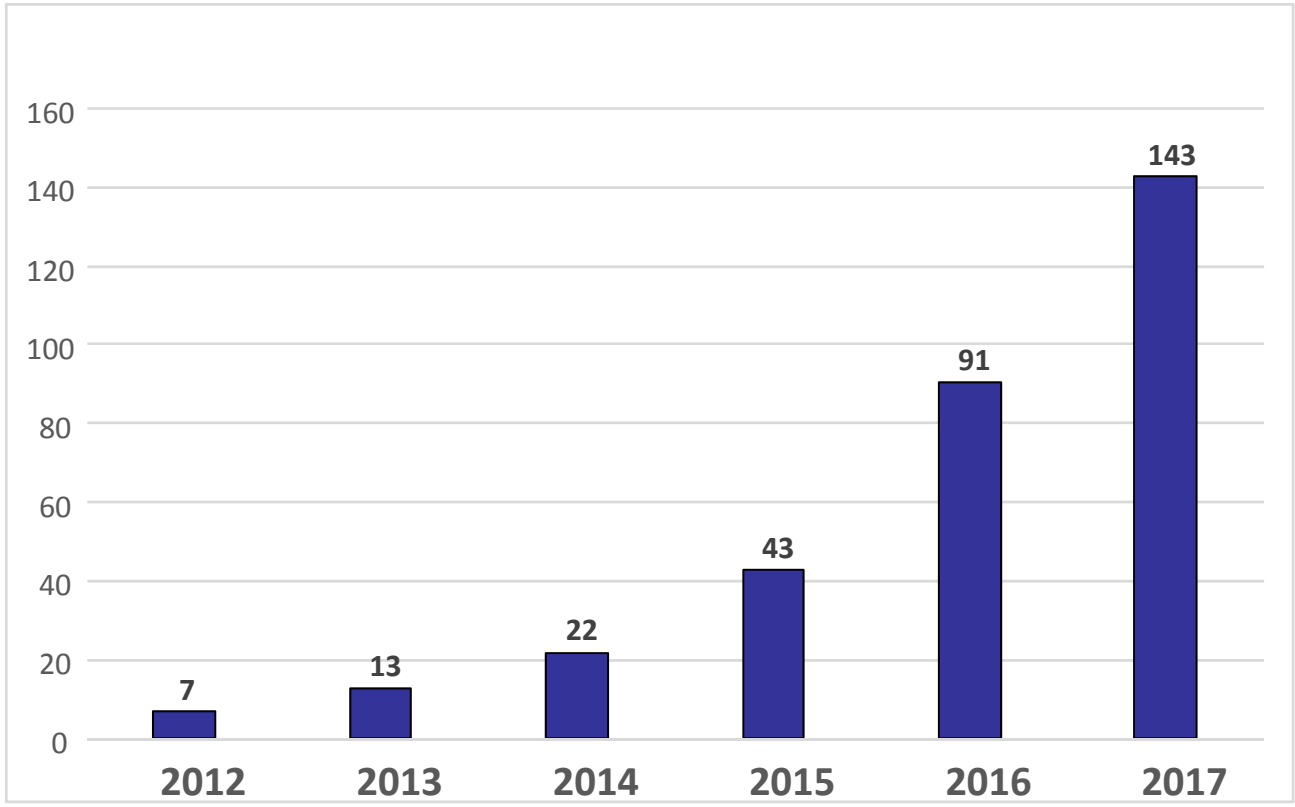
IBV PCR (per month, 2012-2017*)



n=2,672

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IBV in Ontario - Historical

- Sporadic outbreaks
- Respiratory and nephropathogenic field strains described
- Few genotyped but highly related to IBVs circulating in the USA

IBV in Ontario

- Increasing number of IBV cases in early 2012
- Genotype IBVs in samples submitted to the AHL between 2000 and 2013
- Examine genotypes to understand increased IBV cases in Ontario in 2012 and 2013

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Avian Pathology, 2014
Vol. 43, No. 3, 264–268, http://dx.doi.org/10.1080/03079457.2014.916395

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Genotyping of infectious bronchitis viruses identified in Canada between 2000 and 2013

Emily A. K. Martin[1], Marina L. Brash[1], Sarah K. Hoyland[1], Jane M. Coventry[1], Christian Sandrock[1], Michele T. Guerin[2] and Davor Ojkic[1*]

[1]*Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada, and* [2]*Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada*

Infectious bronchitis virus (IBV) was detected in 185 samples originating from chicken flocks of various commodity groups in Canada. Flocks with clinical signs such as respiratory challenge, sudden death, egg production problems, or nephropathogenic conditions, and randomly selected flocks sampled at slaughter as part of an Ontario broiler surveillance project, were included. Most samples were from Ontario and Québec; however, a small number from British Columbia, Nova Scotia, and Newfoundland and Labrador were also analysed. The nucleotide sequence of the spike (S) protein gene was compared with sequences available in GenBank. Based on their S gene sequence similarities, Canadian IBVs could be divided into nine genotypes belonging to four groups: Canadian variant virus, strain Qu_mv; the classic, vaccine-like viruses, Connecticut and Massachusetts; US variant-like virus strains, California 1734/04, California 99, CU_82792, Pennsylvania 1220/98 and Pennsylvania Wolg/98; and non-Canadian, non-US virus, strain 4/91. Based on the field situation, the effectiveness of current vaccination practices mostly based on Massachusetts and Connecticut-type vaccines appeared generally satisfactory for minimizing the damage due to infection with Canadian variant and US variant-like viruses. However, the recent outbreaks of severe respiratory disease and production problems in Ontario chicken flocks related to the incursion of IBV strain 4/91 were not prevented by standard vaccination protocols. It appears that IBV strain 4/91 has now become endemic in Ontario and the need for 4/91-type vaccines must be evaluated.

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Four General Groups

TYPE	IBV STRAIN	SLAUGHTER COLLECTION	CLINICAL SUBMISSIONS	n
Canadian variant	Qu_mv	5	61	66
Classic, vaccine-like	Connecticut	17	10	27
Classic, vaccine-like	Massachusetts	7	19	26
Non-Canadian, non-US variant	4/91	7	21	28
US variant-like viruses	CU_82792	0	14	14
US variant-like viruses	California 1734/04	1	3	4
US variant-like viruses	California 99	0	7	7
US variant-like viruses	Pennsylvania 1220/98	0	9	9
US variant-like viruses	Pennsylvania Wolg/98	0	4	4

Table 1. Genotypes of IBVs in Canada (2000-2013) (n=185)

A survey of the presence of a new infectious bronchitis virus designated 4/91 (793B)

J. K. A. Cook, S. J. Orbell, M. A. Woods, M. B. Huggins

Veterinary Record (1996) **138,** 178-180

On the basis of virus isolation and the demonstration of specific neutralising antibody in sera, infectious bronchitis virus (IBV) 4/91 (commonly called 793B) has been shown to be present in broiler, breeder and layer flocks of chickens in many parts of western Europe and also in Thailand and Mexico. These flocks had all been vaccinated against infectious bronchitis and the need for improved methods to control this new virus, still prevalent at least four years after it was first isolated, is discussed.

IN 1991, outbreaks of what was described as an unusual disease condition were reported in infectious bronchitis-vaccinated breeding flocks of chickens in Great Britain. These outbreaks were shown to be associated with a new and clearly distinct type of infectious bronchitis virus (IBV) called either 793B (Gough and others 1992) or 4/91 (Parsons and others 1992). The unusual features of the infection caused by this virus, here designated 4/91,

TABLE 1: IBV 4/91-specific neutralising antibody in healthy broiler breeder or commercial layer flocks and flocks experiencing infectious bronchitis (IB)-like infections

Country of origin	* Type of bird	Number of flocks with 4/91-specific neutralising antibody Healthy	IB-like infection	Total
Britain	L	1/3 (17-53)†	27/38 (15-69)	28/41
	B	0/4	4/18	4/22
France	L	0/3 (41-45)	4/8 (20-47)	4/11
	B	0/3	3/3	3/6
Netherlands	L	4/5 (20-60)	12/13 (20-60)	16/18
	B	2/4	1/1	3/5
Spain	L	–‡	1/1 (26)	1/1
	B	–	1/2	1/2
Germany	L	1/1 (18)	2/2 (30-35)	3/3
Greece	L	–	5/5 (28-80)	5/5
Mexico	L	–	2/2 (40-48)	2/2
	B	–	1/1	1/1
USA	L	–	0/10 (18-62)	0/10
Thailand	L	–	3/3 (33-64)	3/3

* L Breeder or commercial layer, B Broiler
† Number of flocks positive/number tested (age range of laying birds in weeks)
‡ None tested

IBV 4/91 Geographic Distribution

- Europe: Russia, Slovenia, Hungary, France, Italy, Spain, Netherlands, Germany, Greece, UK
- Africa: Tunisia, Niger, Nigeria
- Asia: India, Iran, Iraq, Japan, China, Jordan, Thailand
- South America: Brazil
- North America: Mexico

IBV in Broilers

4/91

- Age range: 20 to 48 days
- Laying birds affected
- Respiratory
  - Tracheitis,
  - Bronchitis
- Secondary infections
  - pericarditis, perihepatitis, airsacculitis, splenomegaly

IBV (General)

- Young birds most susceptible
- Resistance increased with age
- Respiratory
  - Gasping, coughing, sneezing, tracheal rales, nasal discharge
- Secondary infections (i.e. E. coli)

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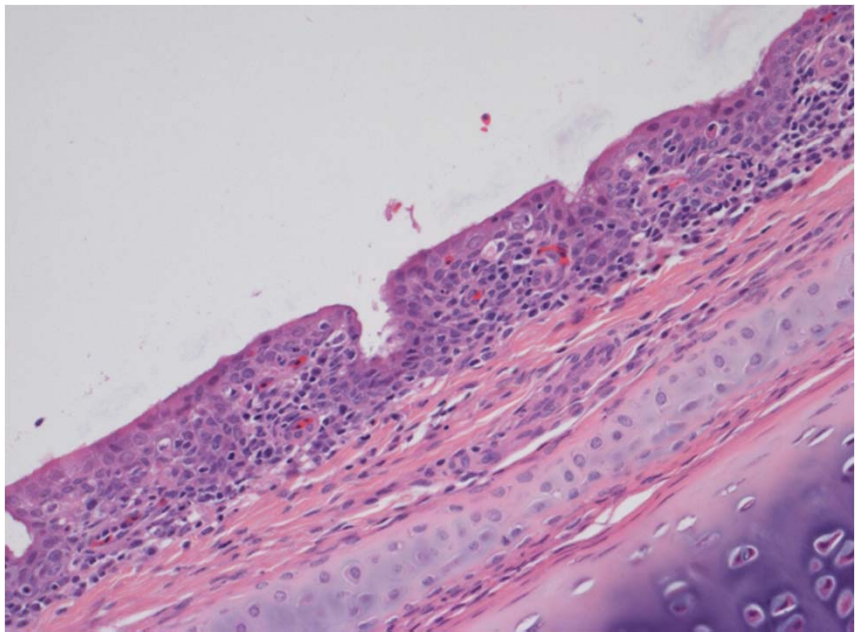
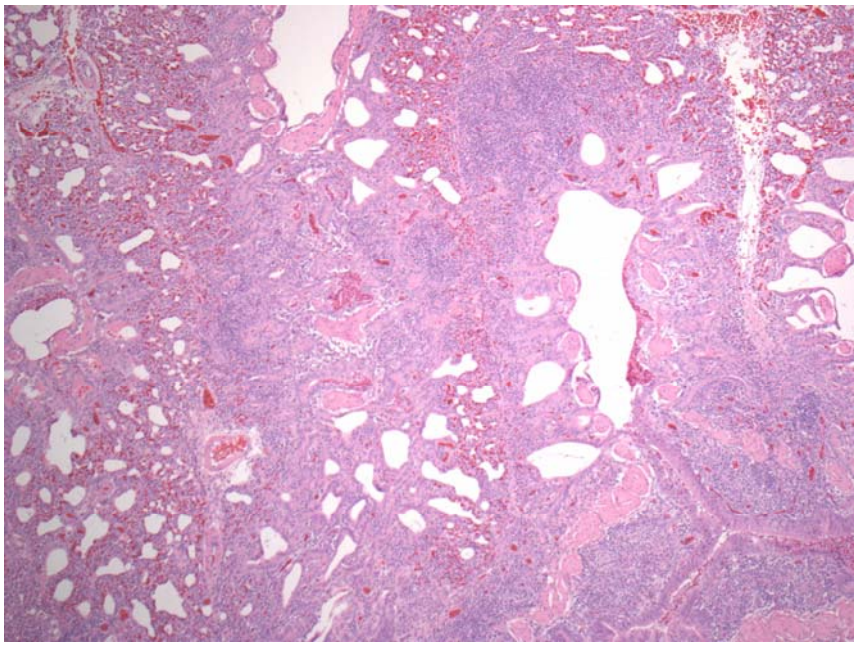
4/91 Histology





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IBV in Broilers

4/91

- Sudden spike in mortality/Sudden death
- High DOAs
- High Condemnations

IBV (General)

- More clinical signs
  - Decreased weight gain and feed efficiency, depression, ruffled feathers, wet droppings, increased water intake, mortality
- Clinically detected prior to shipping

4/91 Clinical Signs

Broiler Breeders: 2 potential presentations

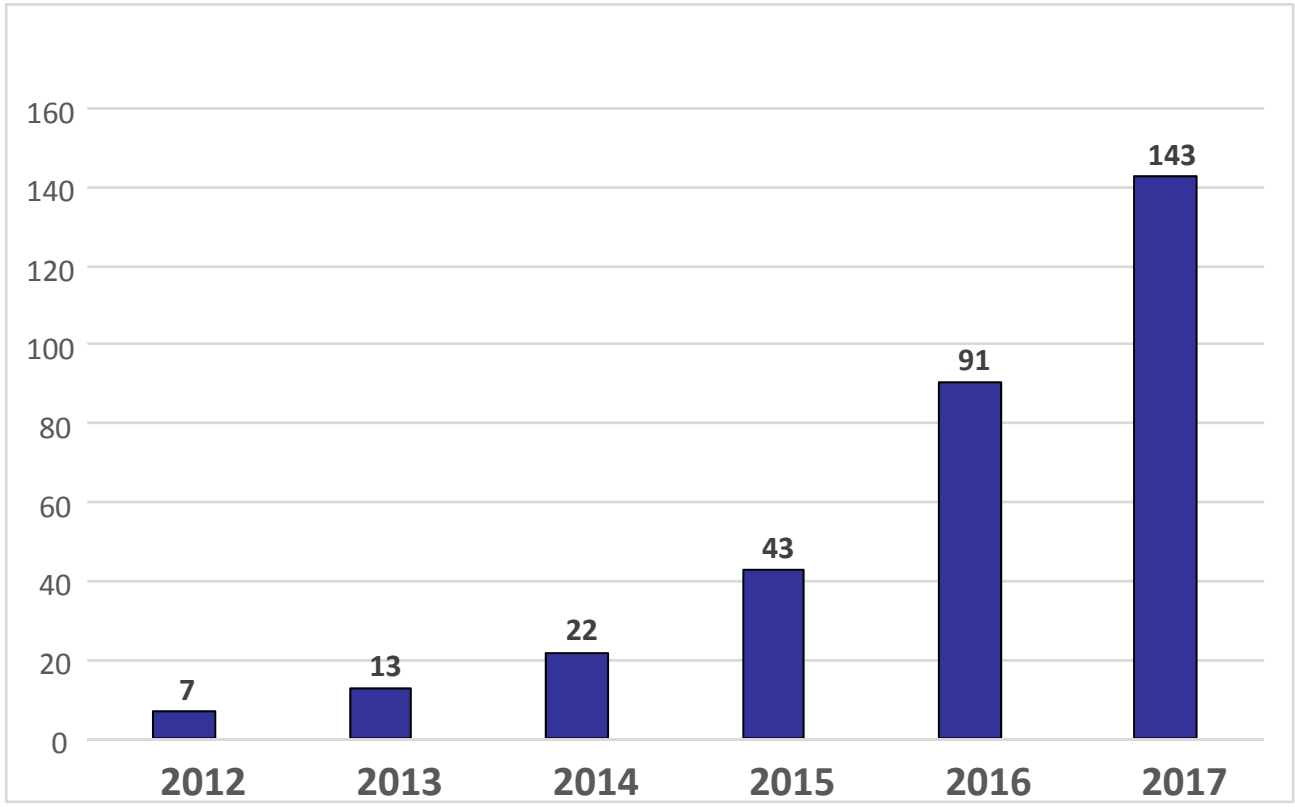
a) Egg quality/mortality issues

- Multi-aged location, moves flock to flock
- Primarily shortly after peak, as young as 22 wks
- Slightly increased mortality
- 3-5% drop in egg production
- white and wrinkled eggs
- 2013-2014 significant mortality & egg drop

b) Hatchability/fertility issues

- Scattered flocks (spiking male associated?)
- After peak to late lay
- 15-30% drop hatch, starts to come back after 6 weeks, on breakout rapid rise infertility.
- Primary type now (increased reports early 2015)

IBV PCR (per month, 2012-2017*)



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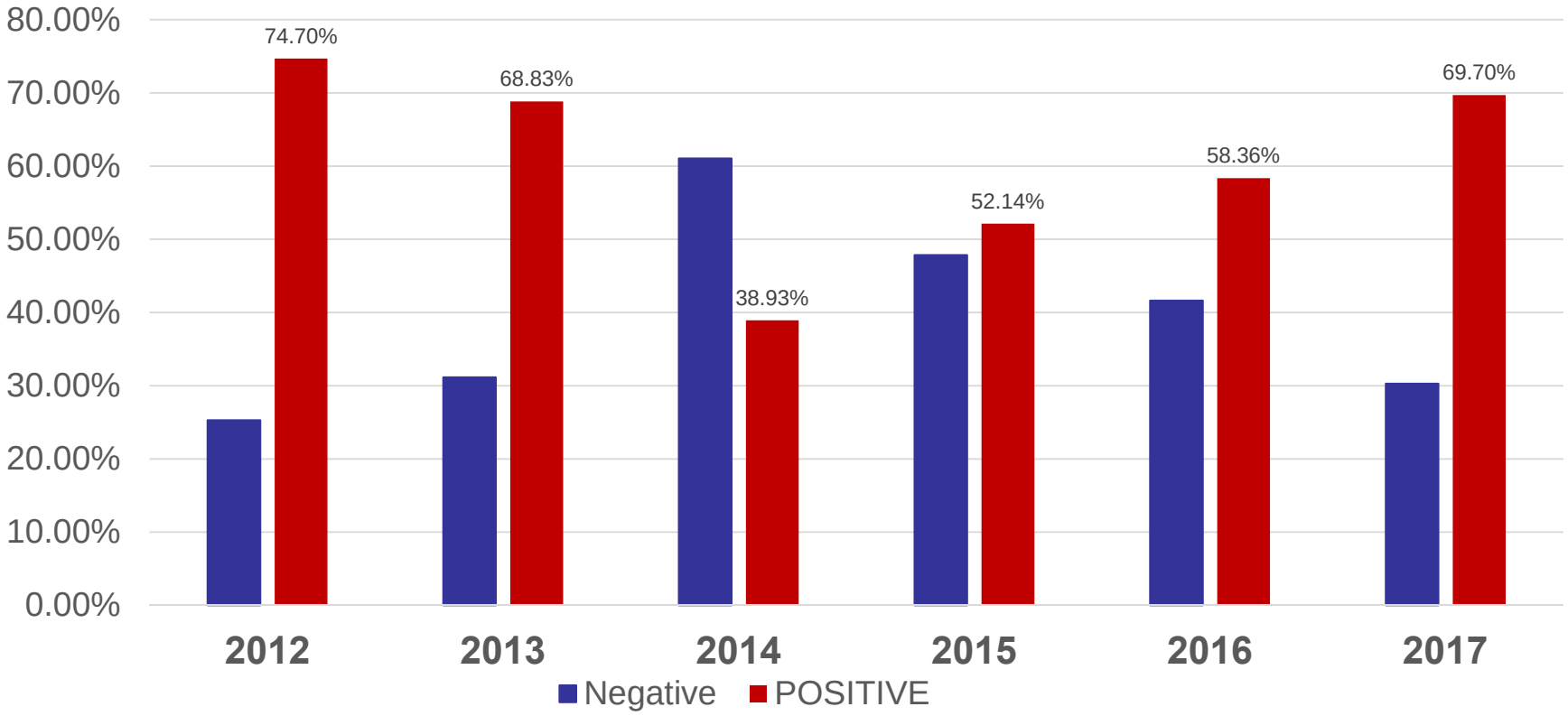
IBV PCR 2012-2017*: RESULTS



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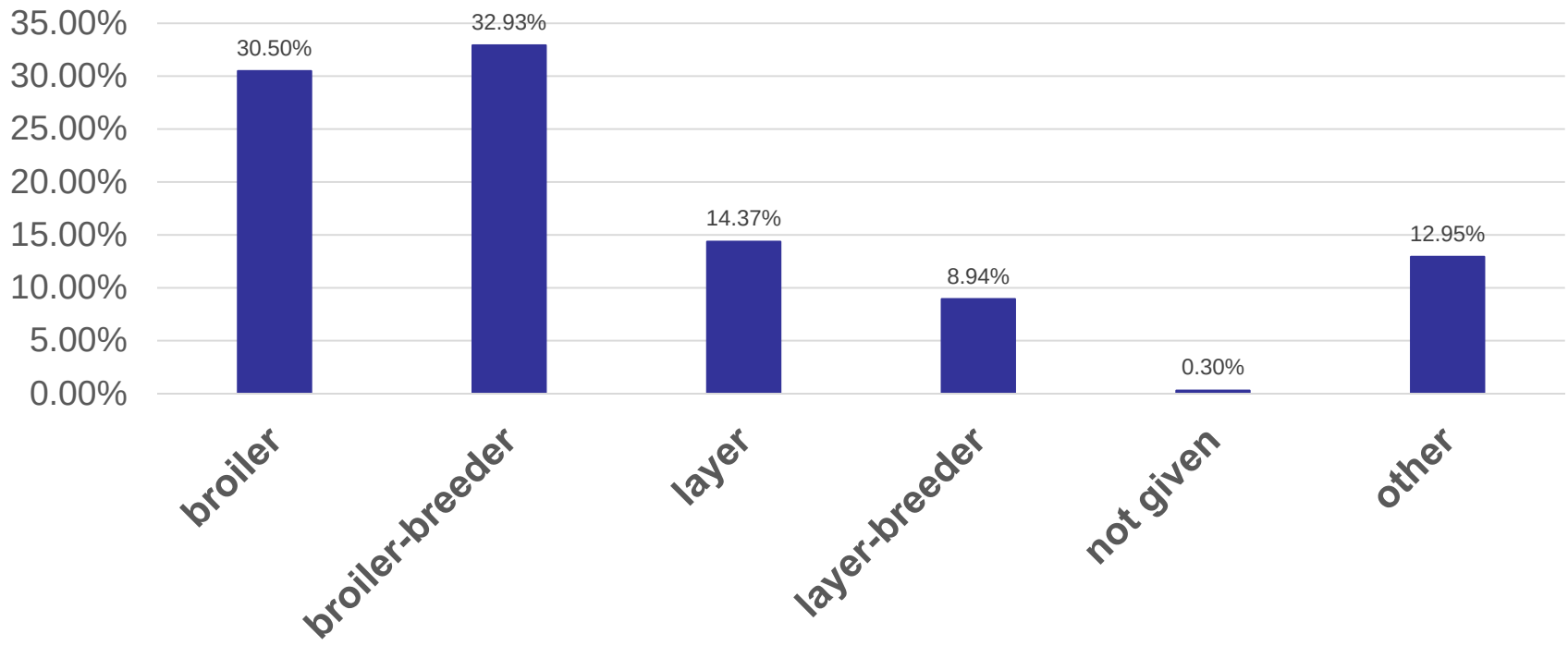
IBV PCR 2012-2017*: COMMODITY GROUPS



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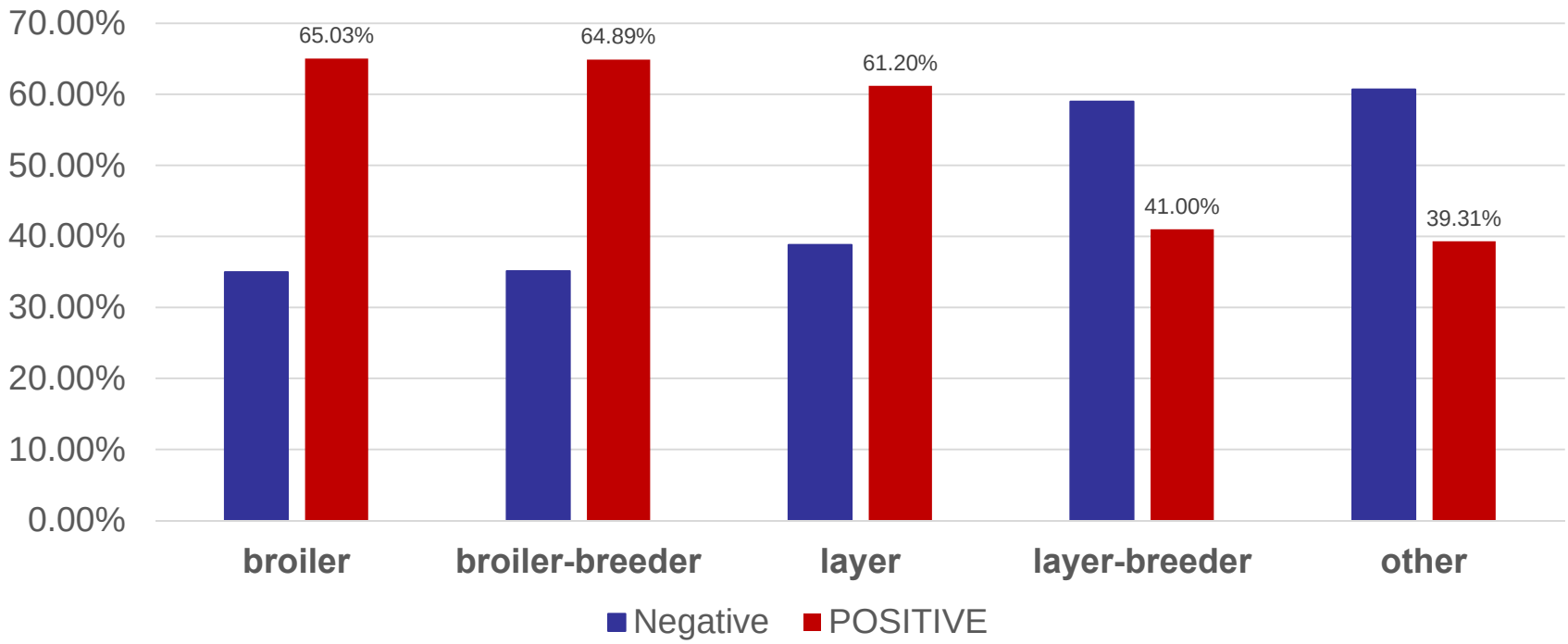
IBV PCR 2012-2017*: RESULT BY COMMODITIES



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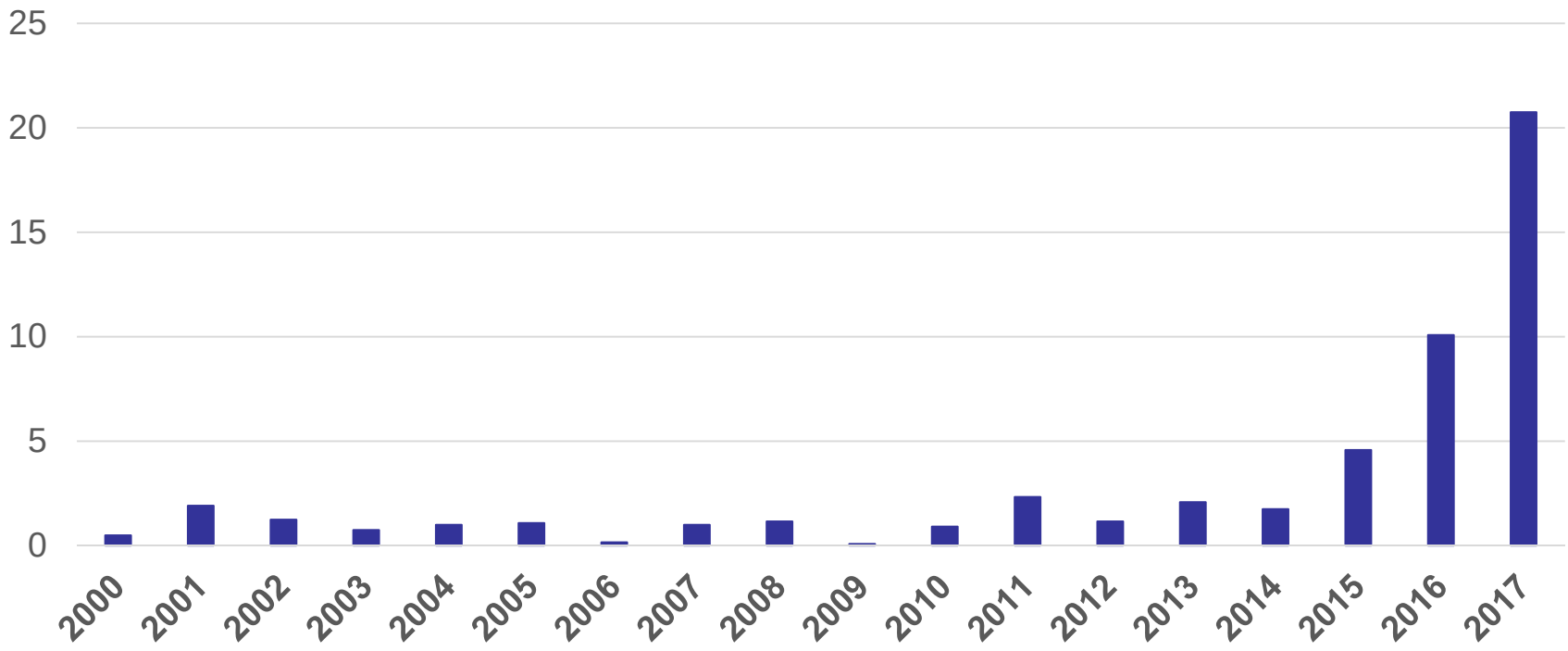
IBV GENOTYPING (per month, 2000-2017*)



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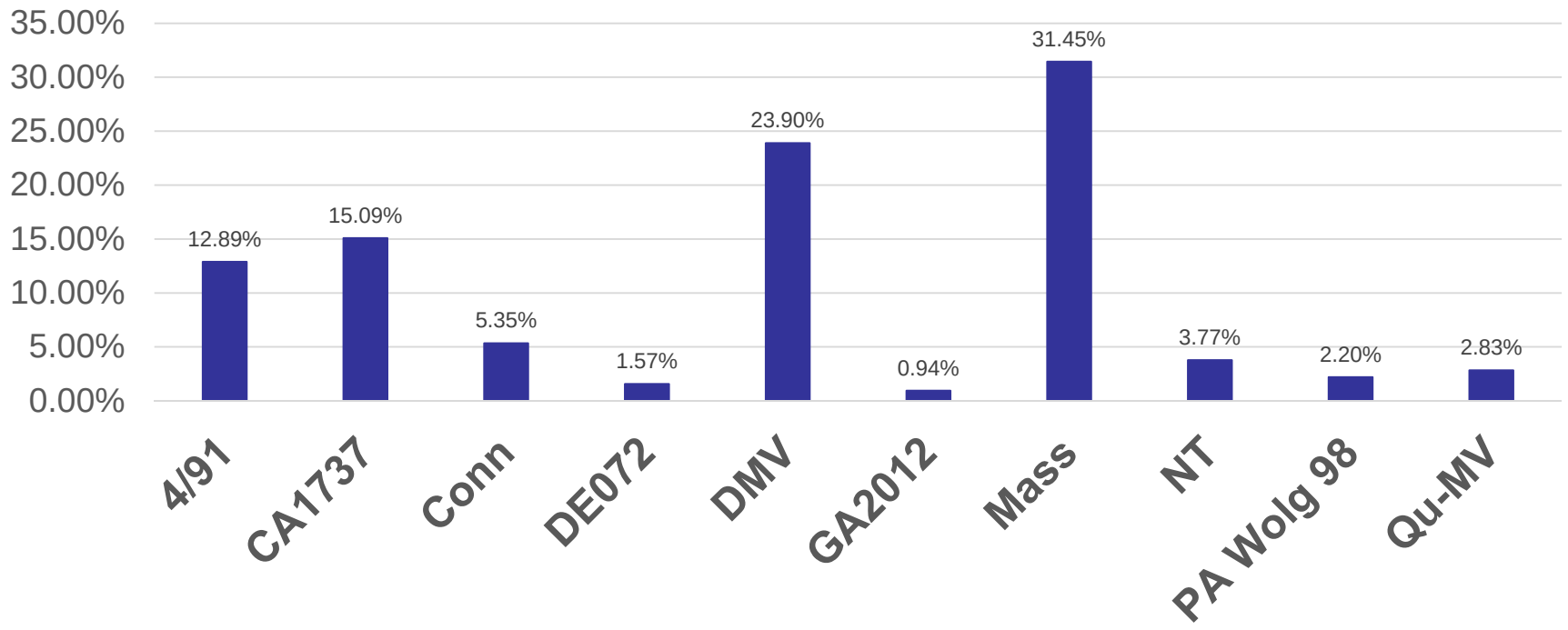
IBV GENOTYPING 2012-2017 (n=318)



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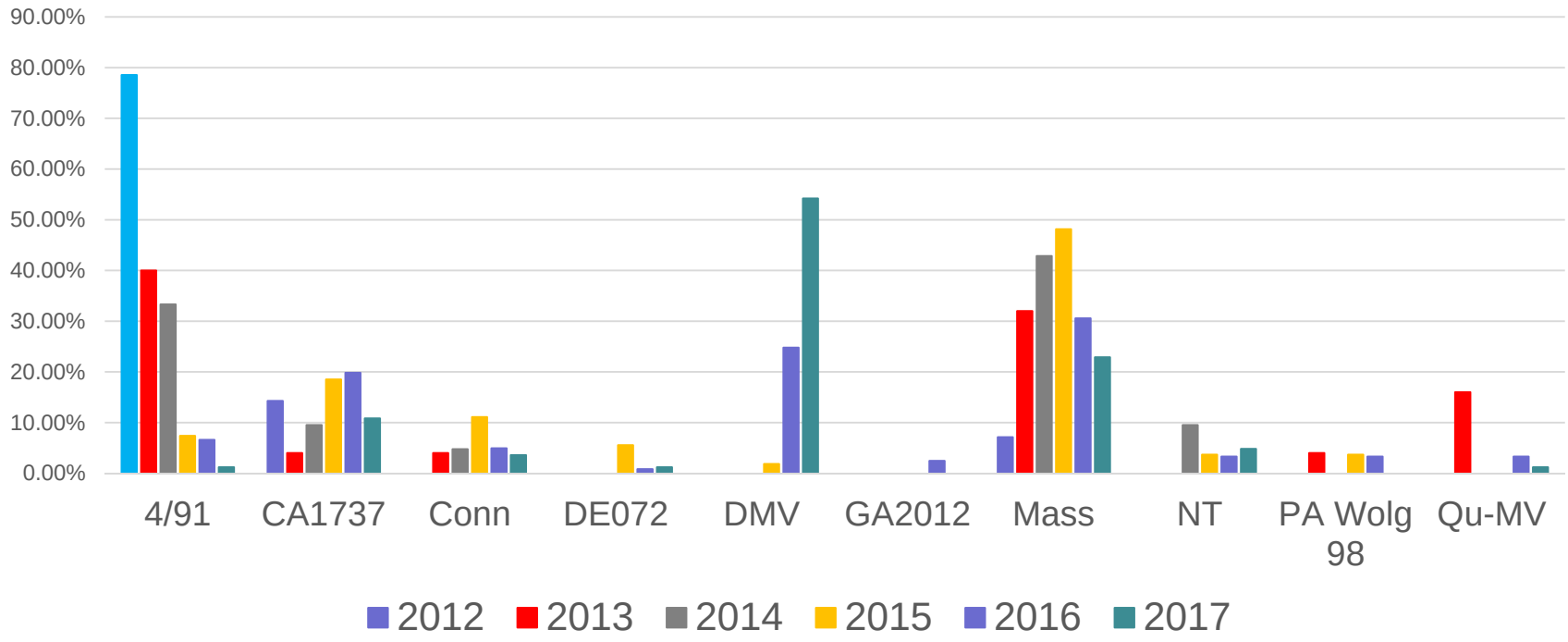
IBV GENOTYPING 2012-2017 (n=318)



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AVIAN DISEASES 57:65–70, 2013

Characterization of Nephropathogenic Infectious Bronchitis Virus DMV/1639/11 Recovered from Delmarva Broiler Chickens in 2011

Jack Gelb Jr.,[AC] Brian S. Ladman,[A] Conrad R. Pope,[A] J. Miguel Ruano,[A] Erin M. Brannick,[A] Daniel A. Bautista,[B] Colleen M. Coughlin,[A] and Lauren A. Preskenis[A]

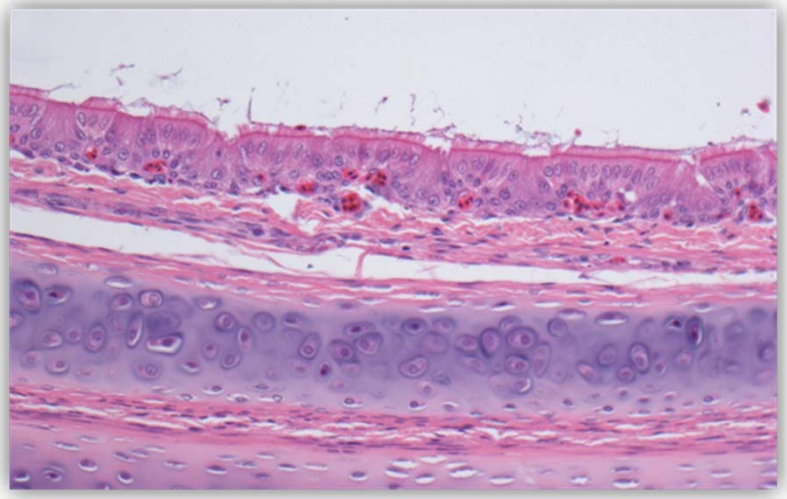
[A]Avian Biosciences Center, University of Delaware, Department of Animal and Food Sciences, Newark, DE 19716-2150
[B]Avian Biosciences Center, University of Delaware, Lasher Laboratory, Elbert N. and Ann V. Carvel Research and Education Center, Georgetown, DE 19947

Received 13 August 2012; Accepted 17 October 2012; Published ahead of print 18 October 2012

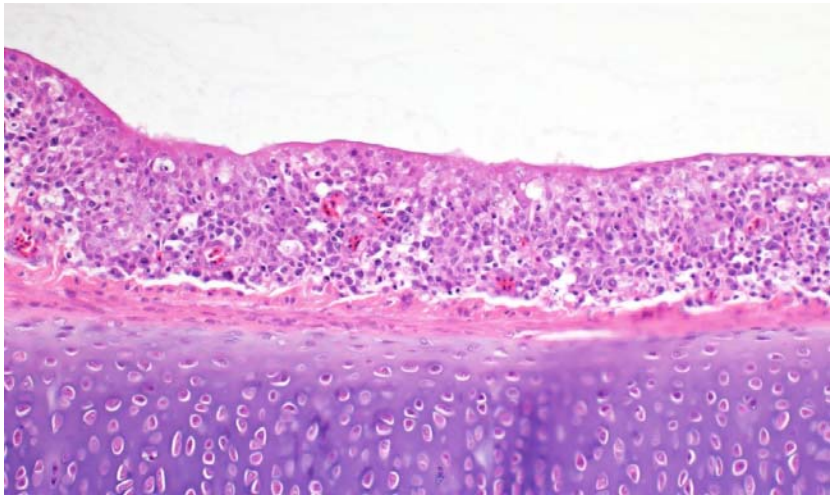
SUMMARY. A limited outbreak of nephropathogenic infectious bronchitis (NIB) occurred in three Delmarva (DMV) commercial broiler chicken flocks in 2011. Isolates of NIB virus (NIBV)—DMV/1639/11, DMV/3432/11, and DMV/3902/11—were characterized by sequence analysis of the N-terminal subunit (S1) of the spike (S) gene. Findings indicated that the isolates were identical to each other and to PA/9579A/10, a 2010 isolate from poultry in Pennsylvania. The 2010 and 2011 isolates appear to have originated from a 1997–2000 NIB outbreak in Pennsylvania. DMV/1639/11 and PA/9579A/10 were determined to be nephropathogenic in susceptible chickens, yielding virus reisolations from kidney and inducing characteristic interstitial nephritis microscopic lesions. In a controlled laboratory study, 40% of chickens vaccinated with a combination live vaccine containing infectious bronchitis virus (IBV) strains Massachusetts (Mass) + Connecticut (Conn) were positive on virus isolation attempts after challenge with DMV/1639/11, compared with only 13% of Mass + Arkansas (Ark) vaccinates. Both combination vaccines gave partial protection against the development of DMV/1639/11-induced renal lesions. Although numerically fewer chickens vaccinated with Mass + Conn had interstitial nephritis compared with those vaccinated with Mass + Ark, neither vaccine combination offered greater protection ($P < 0.05$) than observed in unvaccinated chickens challenged with DMV/1639/11. Mass + Ark vaccinations, applied under commercial conditions in the hatchery (spray) and on-farm (spray), did not protect the trachea or kidney from DMV/1639/11 challenge. Serologic testing of broiler flocks found <3% (2 of 69) tested to possess specific antibodies to DMV/1639/11, indicating the virus had not become established in the region.

DMV (Delmarva)

- Layer, broiler and broiler breeder
- Clinical signs include
  - Very wet litter
  - Hens – small increase in mortality of short duration (~1 week)
  - Decreased egg production of short duration (~ 1 week)
  - Broilers: Increased late bacterial infections, increased ascites, very high bronchitis titres at slaughter, increased condemnations

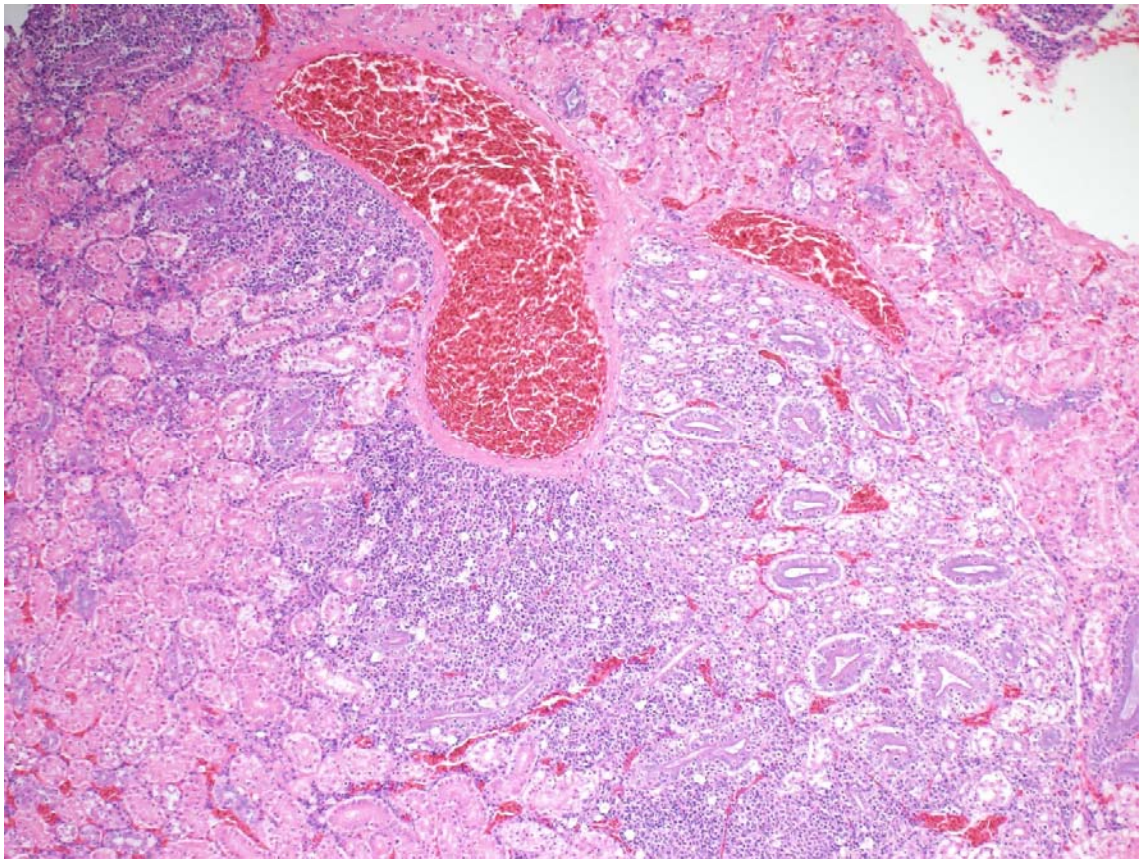


Normal trachea



19-day-old broiler, tracheal pool +ve for DMV strain:

- Marked tracheal mucosal changes
- Flock developed *E. coli* septicemia
- High mortality



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Cevac® IBron Frequently Asked Questions

What is Infectious Bronchitis?

Infectious Bronchitis Virus (IBV) is caused by a chicken coronavirus which is highly contagious and present all over the world. The virus has the ability to replicate and cause lesions in the respiratory tract, kidney and oviduct of the birds, which can lead to increased morbidity and mortality, in addition to decreased egg production and quality in breeders and layers.

The IBV virus is also characterized by its capacity to mutate. Variant IBV strains are continually emerging and breaking through conventional vaccine regimens, creating the need for additional safe and efficacious commercial vaccines.

What is Cevac® IBron?

Cevac® IBron is a live monovalent bronchitis vaccine containing the Georgia (GA) serotype of IBV.

What is unique about Cevac® IBron?

Cevac® IBron offers protection against GA08, GA13 and DMV/1639/11 types of IBV.

What is the recommended use of Cevac® IBron?

Cevac® IBron is recommended for use in healthy chickens one day of age or older by either:

- **Coarse (aqueous) spray:** to aid in the prevention of bronchitis caused by IBV GA08 and aid in the reduction of bronchitis caused by IBV GA13
- **Gel droplet:** to aid in the prevention of bronchitis caused by IBV GA08 and IBV DMV/1639/11

What can I expect from birds vaccinated with Cevac® IBron?

As with any vaccine that has clinical relevance, flocks may experience improved livability and performance (i.e. average daily gain, feed conversion).

What percent protection can I expect from Cevac® IBron?

Efficacy studies were conducted to determine the ability of Cevac® IBron to protect against GA08, GA13 and DMV/1639/11 types of IBV. Cevac® IBron was administered by coarse spray or oral route through gel droplet delivery.

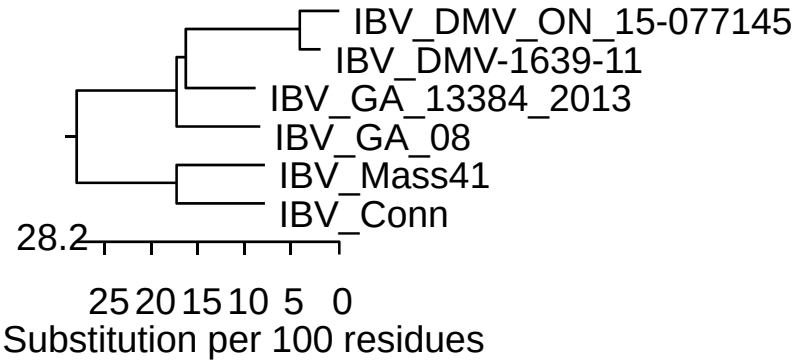
Cevac® IBron protection at 28 days post vaccination

Route	Challenge Virus	% Protection
Coarse Spray	Homologous GA08	90
	Heterologous DMV/1639/11	87
	Heterologous GA13	87
Gel Droplet	Homologous GA08	100
	Heterologous DMV/1639/11	100
	Heterologous GA13	87

Ford, Brianna. "Efficacy Studies of a Variant Infectious Bronchitis Virus Vaccine." AAAP Scientific Program - AAAP 2016 Annual Meeting. American Association of Avian Pathologists. San Antonio Convention Center, San Antonio. 8 Aug. 2016. Poster presentation.

When should the Cevac® IBron vaccine be administered?

Cevac® IBron is recommended for use in healthy chickens one day of age or older.



Percent Identity

	1	2	3	4	5	6		
1		93.7	77.6	79.9	62.9	65.1	1	IBV_DMV_ON_15-077145
2	6.6		79.3	80.5	64.7	65.7	2	IBV_DMV-1639-11
3	26.7	24.3		84.3	70.8	67.1	3	IBV_GA_08
4	23.5	22.7	17.7		69.0	69.5	4	IBV_GA_13384_2013
5	50.9	47.5	37.0	39.9		83.2	5	IBV_Mass41
6	46.8	45.7	43.2	39.2	19.0		6	IBV_Conn
	1	2	3	4	5	6		

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	2012	2013	2014	2015	2016	2017
4/91	78.57%	40.00%	33.33%	7.41%	6.61%	1.20%
CA1737	14.29%	4.00%	9.52%	18.52%	19.83%	10.84%
Conn	0.00%	4.00%	4.76%	11.11%	4.96%	3.61%
DE072	0.00%	0.00%	0.00%	5.56%	0.83%	1.20%
DMV	0.00%	0.00%	0.00%	1.85%	24.79%	54.22%
GA2012	0.00%	0.00%	0.00%	0.00%	2.48%	0.00%
Mass	7.14%	32.00%	42.86%	48.15%	30.58%	22.89%
NT	0.00%	0.00%	9.52%	3.70%	3.31%	4.82%
PA Wolg 98	0.00%	4.00%	0.00%	3.70%	3.31%	0.00%
Qu-MV	0.00%	16.00%	0.00%	0.00%	3.31%	1.20%

SUMMARY

- Increase in submissions related to IBV testing
- Nine IBV types detected since 2012
- Some variant IBVs do not appear to be controlled by Mass/Conn vaccines available in Canada (4/91, DMV)
- Origin of “variant” IBVs
  - Other bird species
  - Other countries
    - 4/91 - Europe, Asia
    - DMV – USA
- Treatment: Management, biosecurity.

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Thank you!

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