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Assessment of the Biological and Genetic Variation of Infectious Laryngotracheitis Virus (ILTV) Isolates from the United States

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"Genetic Differentiation of Field and Vaccine Strains of ILT"

Infectious laryngotracheitis (ILT) continues to cause respiratory disease in many parts of the world including the United States. While the currently available modified live ILT vaccines provide good protection, these vaccine strains may also induce latent infections, and even clinical disease if they are allowed to spread extensively from bird-to-bird in the field. Most ILT researchers in the US believe that vaccine strains have become established in the poultry population by replacing the wild type strains and consequently attribute the prevalence and spread of ILTV induced respiratory disease to improper vac-

Contents

Broiler Live Production Cost	Average Company
Feed Cost/ton w/o color ($)	227.3
Feed cost /lb meat (c)	21.27
Days to 4.6 lbs	42
Chick cost / lb (c)	4.50
Vac-Med cost/lb (c)	0.07
WB & ½ parts condemn. Cost/lb	0.24
% mortality	4.79
Sq.Ft. @ placement	0.81
Lbs/sq. ft.	7.24
Downtime (days)	16

Data for week ending 08 January, 2008

cine application and lax biosecurity. It is however difficult to confirm this because of the apparent genome homogeneity that exists among ILTV field and vaccine strains.

In contrast to other alpha-herpes viral infections where the gene deletion vaccine strains are easily distinguished from field strains, there is no simple way to distinguish ILTV vaccine strains from wild type virus. This has made it impossible to identify the origin of ILTV outbreaks, characterize circulating isolates and perform epidemiological analysis.

It is our hypothesis that in the United States the use of chicken embryo origin (CEO) vaccines in regions densely populated with diverse poultry production units and lax biosecurity, has generated host adapted vaccine-related virus strains that are capable of causing ILT outbreaks when introduced into naïve flocks. In order to test this hypothesis it was first necessary to develop a highly discriminatory, reproducible and portable method to differentiate vaccine strains from field strain isolates based on genetic makeup. Following this it was possible to determine the genetic relationship and biological differences that exist between vaccine strains and the diverse group of US ILTV isolates obtained from ILT outbreaks

Nine viral genotypes (Groups I-IX) were established based on PCR-RFLP and sequencing analysis of the USDA reference strain, vaccine strains and field isolates (Figure 1). PCR-RFLP analysis of multiple genome regions was necessary to precisely differentiate some of the more closely related ILTV isolates. ILTV isolates from commercial poultry grouped separately (III, IV, V and VI), from the backyard flock isolates (VII, VIII and IX).

Regions of diversity in the ILTV genome identified by multiple PCR-RFLP analysis were confirmed by sequencing analysis. With sequencing analysis it was possible to clearly separate commercial poultry isolates into, *vaccine-related isolates* (Groups III, IV, V) and *non vaccine-related* isolates (group VI). Groups IV and V isolates, which were collected from across the United States were also found to be very closely related to the CEO vaccine strains, indicating a widespread prevalence of these genotypes. It is highly likely that these group IV and V isolates are vaccine strains that have lost their attenuation and consequently persist in the field.

Not all the commercial poultry isolates tested were closely related to the vaccine strains. The group VI isolates, present in two states, were genetically very different from the CEO and tissue culture origin (TCO) vaccines. Complete genome sequencing of representative field isolates and vaccine strains will be necessary to precisely determine the origin of these isolates.

Having established that there was significant genetic diversity within the ILT isolates from commercial poultry (genotype groups IV, V, VI) the next step was to determine whether there was an association between viral genotype and phenotypic expression. Pathotyping and cell culture growth studies revealed that some of the viral genotypes had different biological properties both *in vivo* and *in vitro*.

A pathogenicity study performed in specific pathogen free, leghorn type chickens, showed different levels of disease severity and disease duration among the viral genotypes found in commercial poultry. Severity of the disease ranged from moderate to severe depression, moderate to severe conjunctivitis, and various degrees of dyspnea (from open beak to gasping with extended neck). Group IV viruses which are genetically most closely related to the CEO vaccine strain, were equally efficient at replicating in chicken kidney cells, and less pathogenic than group V and VI isolates. These studies suggest that isolates from group IV are not only genetically similar, but share *in vivo* and *in vitro* characteristics with the CEO vaccine strain. Cumulative clinical sign scores indicate that isolates from both genotypes V and VI produced severe depression, severe conjunctivitis and acute respiratory distress, however, the duration of clinical signs differed between these two groups of isolates. Clinical signs cleared at day 10 for group VI isolates, and by day 12 to 14 for group V isolates. With one particular group V isolate, clinical signs lasted up to 14 days and caused the most severe trachea lesions.

In cell culture, group V viruses also produced plaques with the largest diameter when compared to

groups IV and VI viruses, and the CEO vaccine virus. In other alpha-herpesviruses (ILTV viral family) plaque size in cell culture has been correlated with pathogenicity *in vivo*. However, for ILTV a correlation between plaque size and disease severity has not been established.

Through the identification and analysis of a region of the ILTV genome where genetic diversity allows separation and grouping of diverse isolates, it was possible to perform the first comprehensive genotypic analysis of ILT isolates from across the United States. Although biological differences were observed between viral genotypes, the molecular determinants of viral pathogenicity are still unknown. This system of genotypic analysis does however provide a framework for further biological characterization of ILTV isolates providing essential information for the development of effective vaccines for control of this costly disease.

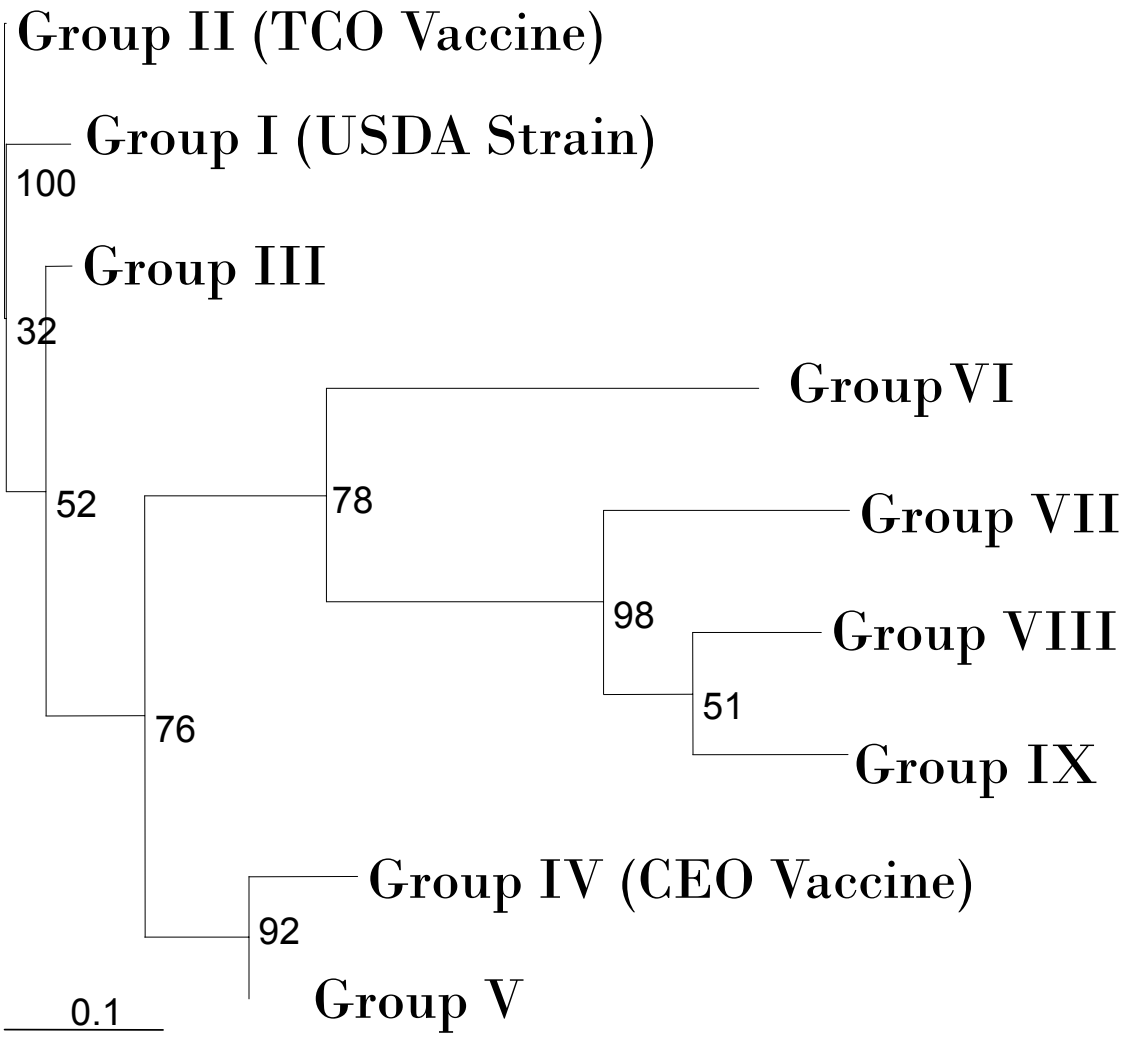

Figure 1: Dendogram based on cluster analysis of PCR-RFLP pattern combination of nine groups of ILTV isolates and strains. The branch lengths represent the genetic distance between the groups, and numbers on the branches are bootstrap values built using the following viruses: Group I: USDA Vaccine Strain; Group II: TCO vaccine strain; Group III: two commercial poultry isolates; Group IV: CEO vaccine strains and six commercial poultry isolates; Group V: nine commercial poultry isolates; Group VI: Five commercial poultry isolates; Groups VII, VIII and IX: three backyard flock isolates.

Exerpts from the latest USDA National Agricultural Statistics Service (NASS) "Broiler Hatchery," "Chicken and Eggs" and "Turkey Hatchery" Report and Economic Research Service (ERS) "Livestock, Dairy and Poultry Situation Outlook"

Chickens and Eggs

Released December 21, 2007, by NASS, Agricultural Statistics Board, USDA

November Egg Production Down 1 Percent

U.S. egg production totaled 7.51 billion during November 2007, down 1 percent from last year. Production included 6.42 billion table eggs, and 1.09 billion hatching eggs, of which 1.02 billion were broiler-type and 66 million were egg-type. The total number of layers during November 2007 averaged 343 million, down 1 percent from last year. November egg production per 100 layers was 2,190 eggs, up 1 percent from November 2006. All layers in the U.S. on December 1, 2007 totaled 343 million, down 2 percent from last year. The 343 million layers consisted of 284 million layers producing table or market type eggs, 56.0 million layers producing broiler-type hatching eggs, and 2.83 million layers producing egg-type hatching eggs. Rate of lay per day on December 1, 2007, averaged 73.1 eggs per 100 layers, up slightly from December 1, 2006.

Egg-Type Chicks Hatched Up 8 Percent

Egg-type chicks hatched during November 2007 totaled 34.0 million, up 8 percent from November 2006. Eggs in incubators totaled 35.9 million on December 1, 2007, up 7 percent from a year ago.Domestic placements of egg-type pullet chicks for future hatchery supply flocks by leading breeders totaled 281 thousand during November 2007, up 36 percent from November 2006.

Broiler-Type Chicks Hatched Up 4 Percent

Broiler-type chicks hatched during November 2007 totaled 761 million, up 4 percent from November 2006. Eggs in incubators totaled 678 million on December 1, 2007, up 4 percent from a year earlier. Leading breeders placed 7.44 million broiler-type pullet chicks for future domestic hatchery supply flocks during November 2007, down 2 percent from November 2006.

Turkey Hatchery

Released December 13, 2007, NASS, Agricultural Statistics Board, USDA

Turkey Eggs in Incubators on December 1 Up 3 Percent from Last Year

Turkey eggs in incubators on December 1, 2007 in the United States totaled 30.3 million, up 3 percent from December 1, 2006. Eggs in incubators were down 3 percent from the November 1, 2007 total of 31.1 million eggs. Regional changes from the previous year were: East North Central down 9 percent, West North Central up 7 percent, North and South Atlantic up 8 percent, and South Central and West down slightly.

Poults Hatched During November Up 8 Percent from Last Year

Turkey poults hatched during November 2007 in the United States totaled 25.8 million, up 8 percent from November 2006. Poults hatched were up slightly from October 2007. Regional changes from the previous year were: East North Central down 1 percent, West North Central up 6 percent, North and South Atlantic up 12 percent, and South Central and West up 14 percent.

Net Poults Placed During November Up 5 Percent from Last Year

The 25.1 million net poults placed during November 2007 in the United States were up 5 percent from the number placed during the same month a year earlier. Net placements were down 1 percent from the October 2007 total of 25.2 million

Broiler Hatchery

Released January 3, 2008, by NASS, Agricultural Statistics Board, USDA.

Broiler-Type Eggs Set In 19 Selected States Up 2 %

Commercial hatcheries in the 19-State weekly program set 220 million eggs in incubators during the week ending December 29, 2007. This was up 4 percent from the eggs set the corresponding week a year earlier. Average hatchability for chicks hatched during the week was 83 percent. Average hatchability is calculated by dividing chicks hatched during the week by eggs set three weeks earlier.

Broiler Chicks Placed Up 5 Percent

Broiler growers in the 19-State weekly program placed 176 million chicks for meat production during the week ending December 29, 2007. Placements were up 5 percent from the comparable week a year earlier. Cumulative placements from December 31, 2006 through December 29, 2007 were 9.14 billion, up 2 percent from the same period a year earlier.

Current Month Charts

Broiler Performance Data Live Production Cost	Region					Average Company
	SW	Midwest	Southeast	Mid-	S-Central	
Feed Cost/ton w/o color ($)	227.76	211.10	230.03	229.59	225.94	227.3
Feed cost /lb meat (c)	21.25	19.21	21.31	22.45	21.44	21.27
Days to 4.6 lbs	42	41	41	41	41	42
Chick cost / lb (c)	4.32	4.46	4.78	3.85	4.28	4.50
Vac-Med cost/lb (c)	0.07	0.03	0.06	0.07	0.05	0.07
WB & ½ parts condemn. Cost/lb	0.21	0.30	0.21	0.25	0.21	0.24
% mortality	4.78	4.71	4.51	5.21	4.35	4.79
Sq.Ft. @ placement	0.78	0.78	0.81	0.89	0.81	0.81
Lbs/sq. ft.	7.36	7.12	7.07	7.70	7.64	7.24
Downtime (days)	15	13	18	17	16	16

Broiler Whole Bird Condemnation	Region					Average Company
	SW	Midwest	Southeast	Mid-Atlantic	S-Central	
% Septox	0.201	0.332	0.185	0.252	0.132	0.216
% Airsac	0.036	0.125	0.131	0.104	0.066	0.088
% I.P.	0.012	0.078	0.009	0.060	0.041	0.034
% Leukosis	0.002	0.002	0.001	0.002	0.001	0.001
% Bruises	0.013	0.002	0.007	0.006	0.004	0.007
% Other	0.010	0.003	0.009	0.008	0.013	0.008
% Total	0.275	0.542	0.340	0.431	0.256	0.354
% ½ parts condemns	0.341	0.401	0.259	0.361	0.375	0.359

Data for week ending 05 January, 2008

Previous Month Charts

Broiler Performance Data Live Production Cost	Region					Average Company
	SW	Midwest	Southeast	Mid-	S-Central	
Feed Cost/ton w/o color ($)	222.08	207.95	221.83	222.39	219.76	220.62
Feed cost /lb meat (c)	20.39	18.76	20.27	21.48	20.61	20.34
Days to 4.6 lbs	42	41	41	40	41	41
Chick cost / lb (c)	4.40	4.44	4.96	3.75	4.22	4.57
Vac-Med cost/lb (c)	0.05	0.02	0.04	0.03	0.03	0.04
WB & ½ parts condemn. Cost/lb	0.20	0.24	0.16	0.20	0.20	0.20
% mortality	4.87	4.48	4.58	4.37	4.29	4.55
Sq.Ft. @ placement	0.78	0.78	0.80	0.92	0.82	0.82
Lbs/sq. ft.	7.10	7.06	6.82	7.56	7.61	7.06
Downtime (days)	17	12	18	17	17	16

Broiler Whole Bird Condemnation	Region					Average Company
	SW	Midwest	Southeast	Mid-Atlantic	S-Central	
% Septox	0.192	0.299	0.124	0.216	0.195	0.2
% Airsac	0.027	0.054	0.058	0.066	0.034	0.046
% I.P.	0.013	0.028	0.006	0.033	0.024	0.020
% Leukosis	0.001	0.001	0.001	0.001	0.000	0.001
% Bruises	0.013	0.002	0.005	0.004	0.004	0.007
% Other	0.010	0.002	0.007	0.003	0.012	0.007
% Total	0.254	0.387	0.201	0.324	0.269	0.281
% ½ parts condemns	0.350	0.379	0.266	0.373	0.367	0.354

Data for week ending December 22, 2007

Meetings, Seminars and Conventions

2008
January

January 23-25: *International Poultry Expo* 2008, Georgia World Congress Center, Atlanta, Georgia. Contact: US Poultry & Egg Association, 1530 Cooledge Road, Tucker, Georgia 30084-7804. Phone: 1-770-493-9401; Fax: 1-770493-9257; email: expogeneralinfo@poultryegg.org.

Website: wv-ww.poultryegg.org or www.ipe08.org

2008
February

Feb 8-12: National Turkey Federation Annual Convention. Loews Coronado Bay, San Diego, California. Details from Adrienne Richards, 1225 New York Avenue, NW, Suite 400 Washington, DC 2005. Phone (202) 898-0100 ext 223, e-mail arichards@turkeyfed.org Website: www.eatturkey.com

2008
March

March 5-6: *Nebraska Poultry industries Annual Convention*, New World Inn & Conference Center, Columbus, Nebraska. Contact: Nebraska Poultry Industries, Inc. University of Nebraska, A103 Animal Sciences, PO Box 830908, Lincoln, Nebraska 68583-0908; Phone: 1-402-472-2051.

March 5-7: *Victam Asia 2008*, Bangkok, Thailand. Contact: Henk van de Bunt, Victam International B.V., P.0. Box 197, 3860 AD Nijkerk, The Netherlands, Phone: +31 33 246 4404, Fax: +3133 246 4706, Email: expo@victam.com; Website: www.victam.com or Contact: Mr. Phusit Sasitaranondha, Thailand, Phone: +66 2 640 8013; Fax: +66 2 664 2076; Email: phusit@expolink.net

March 16-18: 59th North Central Avian Disease Conference: at the St. Paul Rivercentre, St. Paul, Minnesota Dr. Richard Slemons; 1920 Coffey Road; Columbus, OH 43210 or by email slemons.1@osu.edu

March 18-20: 37th annual Midwest Poultry Federation (MPF) Convention in St. Paul, MN. Registration and hotel reservations are available now at www.midwestpoultry.com. Hotel cut-off date is February 11, 2008 and preregistration ends March 1, 2008. Email info@midwestpoultry.com for additional details.

2008
April

April 9-12: **57th Western Poultry Disease Conference**:, 2008 in conjunction with the XXXIII Convención Anual ANECA at the Sheraton Buganvilias Resort, Puerto Vallarta, Jalisco, Mexico. Information can be found at Western Poultry Disease Conference.

April 15-18: Expoviga, Barcelona, Spain. Contact: www.expoaviga.com or expoaviga@firabcn.es

2008
June

June 29—July 4: *XXIII World's Poultry Congress*, Convention and Exhibition Centre, Brisbane, Australia. Contact: WPC 2008 Congress, Interrmedia Convention & Event Management, P.O. Box 1280, Milton, Queensland 4064, Australia,

Phone: +61 7 3858 5594; Fax: +61 7 3858 5510; Email: wpc2008rrim.com.au;

Website: www.wpc2008.com

2008
July

July 6-10: *8th International Marek's Disease Symposium,* Townsville, Queensland, Australia. Contact: Dr. G. Burgess, School of Veterinary & Biomedical Sciences, James Cook University, Townsville, Queensland 4811, Australia.

Phone: +61 7 4781 5472; Fax: +61 7 4781 6833; Email: graham.burgess@jcu.edu.au

July 20-23: American Association of Avian Pathologists annual meeting, New Orleans, Louisiana. Email: aaap@uga.edu

2009
January

January 28-30: *International Poultry Expo* 2009, Georgia World Congress Center, Atlanta, Georgia. Contact: US Poultry & Egg Association, 1530 Cooledge Road, Tucker, Georgia 30084-7804. Phone: +1-770-493-9401; Fax: +1-770-493-9257; Email: expogeneralinfo@poultryegg.org.

Website: www.poultryegg.org

2010
April

April 20-23: *VIV Europe* 2010, Utrecht, The Netherlands. Contact: XNU Exhibitions Europe B.V, P.O. Box 8800, 3503 RV Utrecht, The Netherlands, Fax: +31 302-952-809; Website: www.viv.net

Reminder

All previous issues of the Poultry informed Professional are archived on our website www.avian.uga.edu under the Online Documents and The Poultry Informed Professional links.