

Table S1. Background information of samples from AB provided by Agri Food Laboratories, Alberta Agriculture and Forestry. Samples are arranged by year of submission and the names are color-coded as the phylogenetic tree.

| Sample | Age | Sample Submission Year and Month | Number of birds in the Flock | Number of Birds Affected | Mortality | Clinical Signs | Gross Lesions |
|-------------|--------------|----------------------------------|------------------------------|--------------------------|-----------|--|---|
| AB-S3-ILTV | 6–24 months | oct-13 | 100 | 90 | 15 | Sneezing; frothy eyes; runny nose; rattle breathing; swollen heads; crusting eyes. | Mild catarrhal tracheitis |
| AB-S7-ILTV | Unknown | feb-14 | 28 | 6 | 6 | Lethargic, puffy eyes, gurgling breathing and found dead | Fibrinonecrotizing laryngotracheitis, catarrhal sinusitis, conjunctivitis |
| AB-S11-ILTV | 12–16 months | feb-14 | 50 | 9 | 9 | Sudden deaths | Fibrinonecrotizing laryngotracheitis |
| AB-S13-ILTV | 7 months | jun-14 | 60 | 2 | 2 | Sneezing, watery eyes | Catarrhal sinusitis and tracheitis |
| AB-S15-ILTV | 1.5 months | jun-14 | 250 | 10 | 10 | Lethargy, gasping, gurgling, and neck stretching | Hemorrhagic tracheitis |
| AB-S20-ILTV | 10 months | mar-15 | 150 | 4 | 4 | Puffed-up eyes, difficult breathing, sudden death | Catarrhal laryngotracheitis |
| AB-S23-ILTV | 7–18 months | abr-15 | 40 | 9 | 9 | Sneezing, watery eyes | Tracheitis |
| AB-S31-ILTV | 8 months | abr-15 | 10 | 10 | 1 | Sneezing, cough, raspy breathing | Catarrhal and hemorrhagic tracheitis |
| AB-S35-ILTV | 5 months | sep-15 | 120 | Unknown | 1 | Coughing, bubbly eyes, snotty beak | Catarrhal and hemorrhagic tracheitis |
| AB-S38-ILTV | 4 months | sep-15 | 20 | Unknown | 1 | Lethargy | Mild catarrhal tracheitis |

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|-------------|--------------|--------|---------|---------|-----|---|---|
| AB-S41-ILTV | 18 months | abr-16 | 56 | 22 | 4 | Ruffled feathers, crusty eyes | Hemorrhagic and necrotizing laryngotracheitis |
| AB-S42-ILTV | 15 months | may-16 | 56 | 22 | 4 | Gasping, gurgling and difficult breathing | Fibrino-hemorrhagic and necrotizing laryngotracheitis |
| AB-S44-ILTV | 12 months | sep-16 | 20 | Unknown | 1 | Wheezing respiratory sounds | Catarrhal sinusitis and tracheitis |
| AB-S45-ILTV | 6 months | sep-16 | 50 | 4 | 4 | Puffed-up eyes with ocular secretions, conjunctivitis | Fibrinonecrotizing laryngotracheitis |
| AB-S50-ILTV | 2.5 months | oct-16 | 475 | 400 | 40 | Difficult breathing, neck stretching and gurgling sounds | Fibrinohemorrhagic and necrotizing laryngotracheitis |
| AB-S53-ILTV | Unknown | dic-16 | 30 | 20 | 2 | Breathing difficulties, sneezing | Catarrhal and hemorrhagic tracheitis; catarrhal sinusitis |
| AB-S54-ILTV | Unknown | dic-16 | 30 | Unknown | 2 | Lethargy | Necrotizing laryngotracheitis |
| AB-S55-ILTV | 6 months | dic-16 | 12 | Unknown | 1 | Swollen eyes with discharge and sneezing | catarrhal sinusitis and necrotizing laryngotracheitis |
| AB-S56-ILTV | Unknown | ene-17 | Unknown | Unknown | 8 | Sneezing, respiratory distress, conjunctivitis | Laryngotracheitis, caseous conjunctivitis and sinusitis |
| AB-S62-ILTV | 24–36 months | mar-17 | 100 | 100 | 100 | Breathing difficulties before death | Fibrinonecrotizing tracheitis |
| AB-S61-ILTV | 24 months | mar-17 | 50 | 15 | 15 | Gurgling sounds when breathing | Fibrinonecrotizing laryngotracheitis |
| AB-S63-ILTV | 1.5 months | abr-17 | 150 | 5 | 5 | Nasal and ocular secretions, facial swelling and depression | Fibrinohemorrhagic laryngotracheitis |

| | | | | | | | |
|-------------|------------|--------|-----|----|----|---|--|
| AB-S72-ILTV | 3 months | jun-17 | 70 | 5 | 5 | Breathing difficulties | Mucohemorrhagic laryngotracheitis |
| AB-S77-ILTV | 20 months | sep-17 | 150 | 7 | 4 | Puffy swollen eyes, gasping and neck stretching | Catarrhal and necrohemorrhagic laryngotracheitis |
| AB-S80-ILTV | 7 months | dic-17 | 100 | 21 | 21 | Gasping, runny eyes and sneezing | Fibrinonecrotizing laryngotracheitis |
| AB-S84-ILTV | 5.5 months | dic-17 | 50 | 5 | 0 | Swollen eyes and sneezing | Fibrinonecrotizing laryngotracheitis |
| AB-T85-ILTV | 10 months | ene-18 | 120 | 80 | 80 | Lethargy, swollen eyes and gasping | Fibrinonecrotizing laryngotracheitis |
| AB-S87-ILTV | 24 months | feb-18 | 8 | 2 | 2 | Breathing difficulties | Catarrhal sinusitis and tracheitis |

Table S2. Background information of samples from BC provided by Animal Health Center. Samples are arranged by year of submission and the names are color-coded as the phylogenetic tree.

| Sample | Age | Sample Submission Year | Type of Flock | Type of Operation | Flock Size | Clinical Signs and History |
|----------------|-------------------------|------------------------|---------------|-------------------|---------------|--|
| CAN/BC-9-3204 | 38 days | 2009 | Commercial | Broiler chicken | 7000 | Submitted fresh broiler tissue for ILT by PCR. Mortality of 50 birds. Noticed gasping and mucus discharge on birds. Increased in mortality. Condition suspected: Respiratory. |
| CAN/BC-9-2276 | 12 weeks | 2009 | Commercial | Broiler chicken | 12,000 | |
| CAN/BC-10-1122 | 11 weeks | 2010 | Commercial | Layer chicken | 45,000 | |
| CAN/BC-12-1949 | 36 days | 2012 | Commercial | Broiler chicken | 13,000 | |
| CAN/BC-14-6034 | Between 6 and 10 months | 2014 | Backyard | Layer chicken | Not available | Submitted fresh broiler trachea and lungs for ILT by virology/PCR and histopathology. Postmortem findings: fibrinohemorrhagic exudate in trachea and red congested lungs. Submitted two laying hens for postmortem. All seemed to be healthy. Started losing two birds per week. Sound phlegmy (mucus sounds from throat). Eating and drinking normally. Lost 29 birds in the last 7-10 days |

Table S3. Samples propagated in cell culture and embryonated eggs and number of passages.

| Sample | Method of Propagation | Number of Propagations |
|-------------|-----------------------|------------------------|
| AB-S20-ILTV | CELIC ¹ | 2 |
| AB-S23-ILTV | CAM ² | 2 |
| AB-S45-ILTV | CELIC | 1 |
| AB-S61-ILTV | CAM | 1 |
| AB-S63-ILTV | CELIC | 2 |
| AB-S84-ILTV | CAM | 1 |
| AB-S87-ILTV | CAM | 1 |

¹ CELIC: chicken embryo liver cells. ² CAM: chorioallantoic membrane.

Table S4. Reference strains used in phylogenetic study arranged by country of origin.

| Isolates | Origin | Year | ORF a and b Gene Genotyping | Country | GenBank Accession Number |
|------------------------|-------------------------------|------|-----------------------------|------------------|--------------------------|
| 3.26.90 | Backyard flock | 1990 | IV | USA ¹ | MF417809 |
| 6.48.88 | Backyard flock | 1988 | VII, VIII, IX | USA | MF417810 |
| 14.939 | Broiler | 2014 | V | USA | MF417811 |
| 1874C5 | Broiler | 2004 | VI | USA | JN542533 |
| J2 | Game chickens | 2008 | VI | USA | MF417808 |
| 63140 | Broiler | 2006 | V | USA | JN542536 |
| USDA | Challenge strain | 1960 | I, II, III | USA | JN542534 |
| 81658 | Broiler breeder | 2010 | I, II, III | USA | JN542535 |
| S2.816 | Pea fowl | 2002 | VII, VIII, IX | USA | MF417807 |
| CEO_HPc | CEO ² high passage | 1990 | IV | USA | JN580316 |
| CEO_LPc | CEO low passage | 1990 | IV | USA | JN580317 |
| CEO_TRVX | Vaccine | 1983 | IV | USA | JN580313 |
| Nobilis Laringovac(R) | CEO vaccine | 1975 | IV | USA | KP677881 |
| Laryngo-Vac | CEO vaccine | 1975 | IV | USA | JQ083494 |
| LT-Blen | CEO vaccine | 1975 | IV | USA | JQ083493 |
| TCO_HP | TCO ³ high passage | 1991 | I, II, III | USA | JN580314 |
| TCO_LP | TCO low passage | 1991 | I, II, III | USA | JN580315 |
| TCO-IVAX | Vaccine IVAX | 1983 | I, II, III | USA | JN580312 |
| VFAR-043 | Field Isolate | 2014 | VI | Peru | MG775218 |
| A20 | Vaccine | 1966 | VII, VIII, IX | Australia | JN596963 |
| ACC78 | Broilers/layers | 2008 | IV | Australia | JN804826 |
| CSW-1 | Layer | 1970 | VI | Australia | JX646899 |
| CL9 | Broilers | 2008 | IV | Australia | JN804827 |
| SA2 | Vaccine | 1983 | VII, VIII, IX | Australia | JN596962 |
| SERVA | European CEO vaccine | 2011 | IV | Australia | HQ630064 |
| V1-99 | Layer | 1999 | VI | Australia | JX646898 |
| LJS09 | Layer | 2009 | IV | China | JX458822 |
| K317 | Layer | | IV | China | JX458824 |
| WG | Vaccine | 1950 | IV | China | JX458823 |
| 30678/14/Ko | Commercial flock | | IV | Korea | MH937565 |
| 0206/14/Ko | Commercial flock | | IV | Korea | MH937564 |
| Rus/Ck/Penza/2013/2701 | | 2013 | V | Russia | MF405080 |
| 757/11 | Field Isolate | 2011 | V | Italy | KP677884 |
| 4787/80 | Field Isolate | 1980 | V | Italy | KP677885 |

¹ USA: United States of America. ² CEO: Chicken embryo origin. ³ TCO: Tissue culture origin.

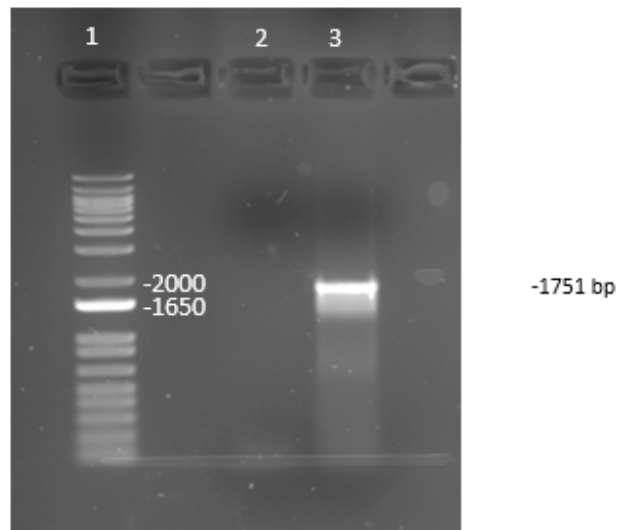


Figure S1. Visualization of PCR product run in a 1% agarose gel of PCR targeting ORF a and b. The amplicon size is 1751 bp (USDA reference genome coordinates 21,703–23,895). The DNA ladder used was 1 kilo base pairs plus (kb+). Lane 1 is the DNA ladder. Lane labeled 2 is the negative control. Lane labeled 3 is a known positive sample to ILTV.