Molecular detection and genetic characterization of infectious laryngotracheitis virus in poultry in Myanmar

CURRENT STATUS: UNDER REVIEW

BMC Veterinary Research  BMC Series

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DOI: 10.21203/rs.2.23718/v1

SUBJECT AREAS
Small Animal Medicine

KEYWORDS
Infectious laryngotracheitis virus, molecular detection, Myanmar, poultry, phylogeny
Abstract
Background Avian infectious laryngotracheitis (ILT) is a highly contagious viral disease that causes severe economic losses to the poultry industry worldwide. In Southeast Asian countries, including Myanmar, poultry farming is a major industry. Although it is known that infectious respiratory pathogens like infectious laryngotracheitis virus (ILTV) are the major threat to poultry farms, there are no data currently available on the epidemiology of ILTV in Myanmar. In this study, therefore, we conducted molecular detection of ILTV in 20 poultry farms in Myanmar.

Results Of the 57 tested oropharyngeal swabs, 10 were positive for ILTV by PCR of a 647 bp region of the thymidine kinase (TK) gene, giving a prevalence of ILTV of 17.5% (10/57). Further sequencing analysis of infected cell protein 4 (ICP4) gene and glycoprotein B, G, and J (gB, gG, and gJ) genes indicated that these isolates were field strains. Phylogenetic analysis revealed that the Myanmar strains clustered together in a single branch and were closely related to other reference strains isolated from Asian countries.

Conclusions This study demonstrated the presence of ILTV in poultry farms in Myanmar. The genetic characterization analysis performed provides the fundamental data for epidemiological studies monitoring circulating strains of ILTV in Myanmar.

Background
Infectious laryngotracheitis (ILT) is an acute and highly contagious viral disease of adult chickens, characterized by inflammation and hemorrhage of the larynx and trachea [1]. The etiological agent is Gallid alphaherpesvirus 1 (GaHV-1), a member of the family Herpesviridae, subfamily Alphaherpesvirinae, genus Iltovirus, and is commonly called infectious laryngotracheitis virus (ILTV) [2]. Acute ILTV infection can cause high morbidity and mortality in chickens while the chronic infection is characterized by decreased growth rates and reduced egg production [3], thus causing serious economic losses to the poultry industry worldwide.

In Southeast Asian countries, including Myanmar, poultry farming is a major industry. Myanmar, which is located in the northeast edge of Southeast Asia, is the largest country in the mainland of Southeast Asia. Along with the increasing demand for sustainable, locally produced, and safe poultry products
for Myanmar consumers, the prevention and control of infectious diseases in poultry has become more important.

Currently, immunization is the principal tool used to control ILTV [4] and the two types of live-attenuated vaccines used are derived from either chicken embryo-origin (CEO) vaccine strains [5], which are attenuated by serial-passage in embryonated eggs, or tissue culture-origin (TCO) vaccine strains [6], which are obtained by multiple passages in tissue culture. However, the vaccine strains can revert to virulence after passages in susceptible birds [7]. Menendez et al indicated that live-attenuated vaccine-related isolates may have contributed to ILT outbreaks worldwide [8]. Therefore, investigating ILTV strains in circulation in endemic areas is not only useful to evaluate vaccine efficacy but also necessary to identify the etiology of disease outbreaks in the poultry population.

Since some ILTV field strains are closely related to the vaccine-derived strains, most studies have used polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) or sequencing a single target region to discriminate between them [9]. However, sequencing multiple target regions would be more useful to better characterize circulating strains and enable more reliable discrimination between ILTV field and vaccine-derived strains [10].

To date, avian influenza and Newcastle disease have been reported in Myanmar [11–13] and recently, the genetic characteristics of other respiratory pathogens, including mycoplasmas and infectious bronchitis virus, have also been investigated [14]. However, to date, there is no scientific report on the epidemiology of ILTV in Myanmar, although some clinical signs suggestive of respiratory pathogen infections have been observed.

In this study, therefore, we aimed to assess the prevalence of ILTV in chickens from major Myanmar poultry farms using molecular detection techniques and perform Sanger sequencing of the isolates to monitor the strains in circulation in this region.

Results
Molecular detection of ILTV in poultry farms of Myanmar

Extracted DNA from pooled swab samples of chickens from 20 poultry farms were subjected to PCR targeting the thymidine kinase (TK) gene. Out of 57 samples collected from different farms, 10
(17.5%) were positive for ILTV (Table 1). The DNA positive control extracted from the ILTV attenuated vaccine showed amplification with a band at the expected size (647 bp) after gel electrophoresis. The nucleotide sequences of the amplified target region were further confirmed by sequencing (data not shown). Of note, most of the positive samples had been collected from the Yangon area during the wet season (May) (Table 1).

Table 1
Details of the distribution of ILTV

| Sampling area | Farm ID | Date            | No. of chickens | No. of detected/No. of tested a (%) |
|---------------|---------|-----------------|----------------|------------------------------------|
| Mandalay      | Ma-1    | Feb. 10, 2018   | 12             | 0/4 (0.0)                          |
|               | Ma-2    | Feb. 10, 2018   | 9              | 1/3 (33.3)                         |
|               | Ma-3    | Feb. 10, 2018   | 9              | 0/3 (0.0)                          |
|               | Ma-4    | Feb. 11, 2018   | 9              | 0/3 (0.0)                          |
|               | Ma-5    | Feb. 11, 2018   | 9              | 0/3 (0.0)                          |
| Pyin Oo Lwin  | Py-1    | Feb. 12, 2018   | 9              | 0/3 (0.0)                          |
|               | Py-2    | Feb. 12, 2018   | 9              | 0/3 (0.0)                          |
|               | Py-3    | Feb. 12, 2018   | 9              | 0/3 (0.0)                          |
|               | Py-4    | Feb. 12, 2018   | 9              | 0/3 (0.0)                          |
|               | Py-5    | Feb. 12, 2018   | 9              | 0/3 (0.0)                          |
| Yangon        | Ya-1    | May 28, 2018    | 9              | 2/3 (66.7)                         |
|               | Ya-2    | May 28, 2018    | 6              | 2/2 (100)                          |
|               | Ya-3    | May 28, 2018    | 6              | 0/2 (0.0)                          |
|               | Ya-4    | May 28, 2018    | 6              | 1/2 (50.0)                         |
|               | Ya-5    | May 29, 2018    | 9              | 3/3 (100)                          |
|               | Ya-6    | May 29, 2018    | 6              | 0/2 (0.0)                          |
|               | Ya-7    | May 29, 2018    | 9              | 0/3 (0.0)                          |
|               | Ya-8    | May 29, 2018    | 9              | 1/3 (33.3)                         |
|               | Ya-9    | May 29, 2018    | 9              | 0/3 (0.0)                          |
|               | Ya-10   | May 29, 2018    | 9              | 0/3 (0.0)                          |

Total | 10/57(17.5) |

a Three samples of the oropharyngeal swabs were pooled and analyzed.

Characterization of the ICP4, gB, gG, and gj genes

To genetically characterize the ILTV isolates, the ICP4, gB, gG, and gj genes were partially amplified in the 10 field samples that were positive for the TK gene. Six samples from different farms (Farm Ma-2, Farm Ya-1, Farm Ya-2, Farm Ya-4, Farm Ya-5, and Farm Ya-8) were selected for sequence analysis; five of the six field samples showed 100% identity with each other although some single nucleotide polymorphisms (SNPs) were also observed in ICP4, gB, gG, and gj genes when compared to reference sequences from GenBank (Table 4 and Table 5). For the ICP4 gene, two fragments located at positions 181–868 and 3645–4268 were used to differentiate ILTV field isolates from live-attenuated vaccine strains as described previously [15]. According to the local poultry farm veterinarians of Myanmar, the TCO-IVAX vaccine was used to control ILTV in these farms. As shown in Table 4, a 12-bp deletion, two
substitutions in the ICP4 gene fragment 1, and five point mutations in the ICP4 gene fragment 2 were observed. These results suggest that all isolates detected in this study were field strains.

Table 4
Nucleotide sequence alignment of ICP4 gene fragments from the isolates in Myanmar, vaccine strains and other ILTV strains

| Name of strains | Nucleotide position from ATG a | ICP4 fragment 1 (positions 181 to 868) | ICP4 fragment 2 (positions 3645 to 4268) |
|-----------------|-------------------------------|---------------------------------------|---------------------------------------|
|                 |                               | 259–270 438 456 594 597 611 795 811 | 3879 3905 3957 3981 4012 4047 |
| Farm Ya-1       | b                             | A A C A A * G G                       | A T C C A A                         |
| Farm Ya-2       | c                             | _ c * _ _ _ _ _ _ _ _               | _ _ _ _ _ _ _ _ _ _               |
| Farm Ya-4       |                              | _ _ _ _ _ _ _ _ _ _               | _ _ _ _ _ _ _ _ _ _               |
| Farm Ya-5       |                              | _ _ _ _ _ _ _ _ _ _               | _ _ _ _ _ _ _ _ _ _               |
| Farm Ya-8       |                              | _ _ _ _ _ _ _ _ _ _               | _ _ _ _ _ _ _ _ _ _               |
| Farm Ma-2       |                              | _ _ _ _ _ _ _ _ _ _               | _ _ _ _ _ _ _ _ _ _               |
| MF41 7811 USA/1  |                              | _ _ _ _ _ _ _ _ _ _               | _ _ _ _ _ _ _ _ _ _               |
| IN542 533       |                               | GCGG G * G G A A                   | - C T - - G                        |
| USDA 874C       |                               | GCGG G * G G A A                   | - C T T G G                        |
| IN542 534       |                               | GCGG G * G G A A                   | - C T T G G                        |
| USDA 1658       |                               | GCGG G * G G A A                   | - C T T G G                        |
| IN542 536       |                               | GCGG G * G G A A                   | - C T T G G                        |
| USA/636 3140    |                               | GCGG G * G G A A                   | - C T T G G                        |
| IN804 827       |                               | GCGG G * G G A A                   | - C T T G G                        |
| Australia/CL 99 |                               | GCGG G * G G A A                   | - C T T G G                        |
| JX646 898       |                               | GCGG G * G G A A                   | - C T T G G                        |
| Australia/A20  |                               | GCGG G * G G A A                   | - C T T G G                        |
| H063 A064       |                               | GCGG G * G G A A                   | - C T T G G                        |
| Australia/Servi |                               | GCGG G * G G A A                   | - C T T G G                        |
| vacci            |                               | GCGG G * G G A A                   | - C T T G G                        |

a: Nucleotide position from ATG
The ICP4 gene sequence with Genbank accession number NC_006623 was taken as a reference.

* Deletions within the sequences.

- Regions where the sequences are identical to those of Farm Ya-1.

| Name of strain | Nucleotide position from ATG<sup>a</sup> |
|----------------|----------------------------------------|
|                | gb | gG | gJ |                 | gb | gG | gJ |                 | gb | gG | gJ |                 | gb | gG | gJ |                 |
| 1931           | C  | G  | C  | T  | 461 | A  | C  | C  | A  | 765 | T  | G  |                 |
| Farm Ya-1      | -  | -  | -  | -  | -   | -  | -  | -  | -   | -   | -  | -  |                 |
| Farm Ya-2      | -  | -  | -  | -  | -   | -  | -  | -  | -   | -   | -  | -  |                 |
| Farm Ya-4      | -  | -  | -  | -  | -   | -  | -  | -  | -   | -   | -  | -  |                 |
| Farm Ya-5      | -  | -  | -  | -  | -   | -  | -  | -  | -   | -   | -  | -  |                 |
| Farm Ya-8      | -  | -  | -  | -  | -   | -  | -  | -  | -   | -   | -  | -  |                 |
| Farm Ma-2      | -  | -  | -  | -  | -   | -  | -  | -  | -   | -   | -  | -  |                 |
| MF41           | -  | A  | G  | -  | -   | -  | -  | -  | T   | -   | -  | -  |                 |
| 7811 USA       | -  | -  | -  | -  | -   | -  | -  | -  | T   | -   | -  | -  |                 |
| 14.9           | -  | -  | -  | -  | -   | -  | -  | -  | T   | -   | -  | -  |                 |
| IN54/2533 USA  | -  | G  | A  | -  | -   | -  | -  | -  | T   | -   | -  | -  |                 |
| Accession | Country | Identity | Sequences | Variant |
|-----------|---------|----------|-----------|---------|
| IN54 2534 | USA     | 187      | A         | T       |
| IN54 2535 | USA     | 7816     | A         | T       |
| IN54 2536 | USA     | 7631     | A         | T       |
| IN80 4827 | Australia | V1-99    | A         | T       |
| JN59 6963 | Australia | vaccine | A         | T       |
| HQ6 3006  | Australia | vaccine | A         | T       |
| JX45 8822 | China    | vaccine | A         | T       |
| JX45 8823 | China    | vaccine | A         | T       |
| JX45 8824 | China    | vaccine | A         | T       |
| MH9 3756  | Korea    | vaccine | A         | T       |
| MH9 3756  | Korea    | vaccine | A         | T       |
| MH9 3756  | Korea    | vaccine | A         | T       |
| JN58 0312 | TCO      | vaccine | A         | T       |
The sequence of each gene (gB, gG and gJ genes) with Genbank accession number NC_006623 was taken as a reference.

- Regions where the sequences are identical to those of Farm Ya-1.

In this study, the SNP at position 1931 in the gB genes from the field isolates, except for Farm Ya-5, was cytosine, similar to what is seen in most field strains, whereas the one from most vaccine strains coded for thymine (Table 5). This point mutation led to an isoleucine-to-threonine substitution at position 644 (I644T) in the gB protein of field strains. Similarly, some SNPs were observed in the gJ and gG genes of ILTV. The sequences of the gJ gene fragments from the Myanmar isolates, except for Farm Ya-5, were identical to those from a China/K317 vaccine-derived strain and a Korean field strain, whereas the sequences of the gG gene fragments from the Myanmar isolates were unique (Table 5). Moreover, a point mutation in position 102 in the gG gene led to a non-synonymous amino acid substitution (Glutamic acid-to-Aspartic acid substitution at position 34, E34D).

Phylogenetic analysis of the ICP4, gB, gG and gJ genes

Phylogenetic analysis based on the ICP4 and gB genes showed that five out of six isolates obtained in this study clustered together and were closely related to reference strains, including from Asian countries (Fig. 1a and Fig. 1b). In contrast, the phylogenetic tree constructed using the gG and gJ genes showed that the five isolates in Myanmar formed into a distinct cluster, separate from other reference strains deposited in the GenBank database (Fig. 1c and Fig. 1d). Altogether, these results suggest that the ILTV isolates detected in poultry farms in Myanmar might be similar to those circulating in neighboring Asian countries, and perhaps been endemic for a certain time given the presence of the unique mutations in the gG and gJ genes.

Discussion

Although ILTV causes less mortality than the highly pathogenic avian influenza virus and Newcastle disease, its
impact on avian productivity has caused significant economic losses to the poultry industry worldwide [16]. However, no scientific data on ILTV surveillance in poultry farms in Myanmar was available until now. In this study, we investigated the presence of ILTV in Myanmar among 20 poultry farms in Myanmar using PCR targeting the TK gene and we detected ILTV in six farms which were located in southern Myanmar. Molecular characterization of ILTV is required to differentiate between field and vaccine strains [15,17,18]. ICP4 is responsible for regulation of gene expression early in infection [19] and has been proposed as a potential differentiation marker due to differences in this gene in the wild-type and vaccine strains [20]. The sequences from the isolates in Myanmar in the present study had a 12 bp-deletion at positions 259–270 in the ICP4 gene fragment 1; this deletion is typically not present in the TCO vaccine strains. In addition, the nucleotide sequences of ICP4 gene fragment 2 in the isolates showed distinct differences from TCO vaccine strain sequences. According to the local veterinarians from Myanmar poultry farms, TCO vaccine strain is used to prevent the incidence of ILT in poultry farms that we visited. Therefore, the isolates detected in the present study appear to be field strains. Glycoprotein B encoded by UL27 gene is one of the major proteins in ILTV, playing a fundamental role in virus attachment to target cells and cell entry [21]. According to our data, the point mutation at position 1931 in the gB gene was found in most virulent and vaccine strains (including TCO and CEO strains). Gracía et al. also reported that the codon at position 1931 in the gB gene from most field strains coded for cytosine whereas the one from most vaccine strains coded for thymine [22]. Therefore, the SNP at position 1931 in the gB gene could act as a good differentiation marker for field and vaccine strains [9,22]. In contrast, the isolate from Farm Ya-5 showed similarity to the vaccine strains as well as a few field isolates. gJ protein is a major viral antigen and plays an important role during egress of ILTV [23]. Craig et al. [1] compared seven different partial fragments of some ILTV genes (TK, gD, gG, gB, gC, gJ, and ICP4), and indicated that the gJ sequence was the most informative segment to discriminate between field and vaccine strains. Furthermore, five distinct haplotypes were defined according to the specific changes in select nucleotide positions of the gJ gene. Sequence analysis in the present study showed that haplotype 2 was the predominant type circulating in Myanmar (data not shown). Sequencing analysis of the gG gene has also been used to characterize ILTV isolates [24]. By comparing the partial sequence of gG genes with those of other reference strains, a non-synonymous substitution (Glu-to-Asp) at
position 34 was identified in the gG gene of field isolates from this study. To our knowledge, no other studies have reported this mutation in the gG gene of either field or vaccine strains. Further investigation of ILTV strains circulating in the other regions of Myanmar is therefore necessary. Furthermore, since ILTV gG is a known virulence factor that can bind chemokines with high affinity and inhibit leukocyte chemotaxis [25, 26], the biological significance of this amino acid substitution (Glu 34 Asp) in the gG gene requires further investigation to determine whether it impacts on the pathogenicity of ILTV.

In the present study, ILTV was mainly detected in the Yangon farms (southern area of the country). All the Yangon samples were collected in May, which is the wet season in Myanmar. In contrast, the Mandalay and Pyin Oo Lwin samples were collected in February, which is the dry season, and almost all were negative for ILTV. The climate during the dry season is much warmer than during the wet season in Myanmar. Since ILTV is a temperature-sensitive virus that cannot resist high environmental temperatures, it is possible that ILTV transmission may be limited during the dry season, thus partially explaining why most positive samples were detected from Yangon farms and very few from Mandalay and Pyin Oo Lwin farms. Therefore, future studies should ensure that sampling is conducted during similar seasons to ensure accurate representation of the circulating ILTV strains in Myanmar.

Phylogenetic analyses of the ICP4 and gB genes indicated that the Myanmar ILTV isolates were closely related to ILTV reference strains including Asian strains, especially three Korean field isolates, which most likely originated from the Serva vaccine strain [27]. According to the phylogenetic analysis comparing the gB and gG gene sequences obtained in this study and those previously published in Genbank, five Myanmar isolates clustered into separate branches belonging to the CEO vaccine and TCO vaccine strains. In contrast, phylogenetic analysis using the gJ and ICP4 gene sequences revealed that these isolates clustered together with CEO vaccine. In a previous study by Oldoni et al [28], three isolates could only be differentiated from the CEO vaccine by the analysis of glycoprotein M gene. Meanwhile, molecular techniques have identified live-attenuated vaccines as one of the main causes of ILTV outbreaks worldwide [8]. CEO vaccine has been banned in Argentina for more than 10 years due to its associated reversion to virulence [1]. Shehata et al. [29] also isolated three highly pathogenic CEO-like field strains and suggested that CEO vaccine strains could increase in virulence after bird-to-bird passages causing severe outbreaks in susceptible birds. It is more likely that the ILTV isolates circulating in poultry farms in
Myanmar originated from CEO-like viruses. However, such a hypothesis requires further periodical surveillance using larger sample sizes and sequence analysis based on additional ILTV genomic regions.

Conclusions
This study demonstrated the presence of ILTV in poultry farms in Myanmar. Genetic characterization of the ICP4, gB, gG, and gj genes indicated that these isolates were different from vaccine strains and seemed to be field strains circulating in Myanmar. Phylogenetic analysis revealed that these isolates clustered together in a single branch and were closely related to other reference strains, in particular Asian isolates. These results provide some fundamental data for epidemiological studies monitoring the spread of ILTV in Myanmar.

Materials And Methods
Sample collection
Sample collection was conducted as previously reported at 20 chicken farms located in three major poultry-farming areas in Myanmar, namely Mandalay, Pyin Oo Lwin, and Yangon [14]. Briefly, oropharyngeal swabs were collected from five farms in Mandalay and five farms in Pyin Oo Lwin in February 2018, and ten farms in Yangon in May 2018. In each farm, swab samples were collected from six, nine, or twelve adult laying hens whose breeds were Rhode Island Red or White Leghorn (Table 1). All samples were transferred to the laboratory at 4 °C within 2 days of swab collection and were then stored at -80 °C until use.

DNA extraction and molecular detection of ILTV
Three swab samples were pooled and DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The extracted DNA samples were stored at -20 °C until use.

The thymidine kinase (TK) gene of ILTV was targeted for the detection of ILTV by PCR using previously published primers (Table 2) [30]. The PCR mixture contained 10 pmol of each primer, 1 U of TaKaRa Ex Taq (TaKaRa Bio Inc., Kusatsu, Japan), and 200 µM of each deoxynucleotide (TaKaRa Bio Inc.). The DNA sample obtained from an attenuated ILTV live vaccine (LT-IVAX strain) (Kyoritsu Seiyaku Corporation) was used as a positive control.

Negative controls, namely the PCR master-mix alone and ultrapure water without DNA, were included with each PCR run.
Table 2
Primers used for amplification of each gene in this study

| Target gene | Primer name | Primer sequences (5' - 3') | PCR conditions | Expected size (bp) | References |
|-------------|-------------|----------------------------|----------------|-------------------|------------|
| For detection of pathogen |TK| TK-F | ACG ATG ACT CCG ACT TTC| 94℃ 2 min; 35 × (94℃ 30sec, 55℃ 30sec, 72℃ 50sec); 72℃ 10 min | 647 | Pang et al.[30] |
| |TK-R | CGT TGG AGG TAG GTG GTA| | | |
| For sequence analysis |gB| gB-F | CAA GGG CGG AAT TTG ATA GA| 94℃ 2 min; 35 × (94℃ 30sec, 55℃ 30sec, 72℃ 50sec); 72℃ 10 min | 440 | This study |
| |gB-R | AAT GAG GCG ATG CCA GAT GC| | | |
| |gG| gG-F | TTG TGC GCG TCT GTA TTA GG| 94℃ 2 min; 35 × (94℃ 30sec, 55℃ 30sec, 72℃ 30sec); 72℃ 10 min | 612 | This study |
| |gG-R | CTC CAT AGG ACC GTC GAG TT| | | |
| |gJ| gJ-F | GTT AAC GCC TCT CGT GAA CG| 94℃ 2 min; 35 × (94℃ 30sec, 55℃ 30sec, 72℃ 50sec); 72℃ 10 min | 667 | This study |
| |gJ-R | TCG GGG AAG TAC CTG TAT CG| | | |
| |ICP4 fragment 1| ICP4a-F | ACT GAT AGC TTT TCG TAC AGC ACG| 94℃ 2 min; 35 × (94℃ 30sec, 55℃ 30sec, 72℃ 50sec); 72℃ 10 min | 688 | Chacon et al. [15] |
| | | ICP4a-R | CAT CGG GAC ATT CTC CAG GTA GCA| | | |
| |ICP4 fragment 2| ICP4b-F | CGA AAT CGG AAA AGC TTC AG| 94℃ 2 min; 35 × (94℃ 30sec, 55℃ 30sec, 72℃ 50sec); 72℃ 10 min | 624 | This study |
| | | ICP4b-R | CTC CAG CAA CAA CAC ATT GG| | | |

Genetic characterization of ILTV

DNA samples positive for TK gene were subjected to sequencing analysis of ICP4, gB, gG, and gJ genes. For each gene, the regions in which the polymorphisms are typically observed among ILTV strains were partially amplified by PCR (Table 2). For sequencing, the amplified DNA products were purified using a FastGene gel/PCR extraction kit (NIPPON Genetics Co. Ltd.), and the nucleotide sequences were determined using the GenomeLab™ GeXP Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA). The obtained sequences of the ICP4, gB, gG, gJ genes were aligned with reference sequences from GenBank database (Table 3) using MEGA6 software [31] and the phylogenetic trees were generated with the same software using the neighbor-joining method coupled with Kimura 2-parameter model with bootstrap analysis of 1000 replicates [32].
Table 3
Reference strains used in this study

| Virus strains | Origin      | Country | Accession |
|---------------|-------------|---------|-----------|
| 14.939        | Field strain | U.S.    | MF417811  |
| 1874C5        | Field strain | U.S.    | JN542533  |
| USDA          | Challenge strain | U.S.    | JN542534  |
| 81658         | Field strain | U.S.    | JN542535  |
| 63140         | Field strain | U.S.    | JN542536  |
| CL9           | Field strain | Australia | JN804827 |
| V1-99         | Field strain | Australia | JX646898 |
| A20           | Vaccine strain | Australia | JN596963 |
| Serva         | Vaccine strain | Australia | HQ630064 |
| LJS09         | Field strain | China    | JX458822  |
| WG            | Field strain | China    | JX458823  |
| K317          | Vaccine strain | China    | JX458824  |
| 0206/14/Ko    | Field strain | Korea    | MH937564  |
| 30678/14/Ko   | Field strain | Korea    | MH937565  |
| 40798/10/Ko   | Field strain | Korea    | MH937566  |
| TCO-IVAX      | Vaccine strain | U.S.    | JN580312  |
| CEO-TRVX      | Vaccine strain | U.S.    | JN580313  |
| Gallid herpesvirus 1 | N/A               | N/A     | NC006623 a |

a The ILTV DNA sequence was assembled from 14 published ILTV sequences \[33\].

N/A: Not applicable

Declarations

Ethics approval and consent to participate

Swab samples used in this study had obtained based on informed consent from farm owners. Collection of swab samples from chickens was approved by the Ministry of Agriculture, Livestock and Irrigation of Myanmar (approval number: 5/6000/moali (1192/2017) and 1080/pa10(kha)/2017).

Consent for publication

Not applicable.

Availability of data and material

The datasets supporting the conclusions of this article are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding
This research was supported in part by Grants-in-Aid for Scientific Research (B: 16H05804 and B: 18H02332) and a Young Scientists grant (B: 16K18798) from the Japan Society for the Promotion of Science. All of the funding bodies were applied to sample collection, data analysis and interpretation, and writing the manuscript.

Authors’ contributions
ZY, SM, and SF were responsible for the conception and design of the study. SM, MT, KK, MMH, and SYW collected samples. MMH, SYW, and SB obtained the informed consent from farm owners before the sample collection, and performed all the procedures for sample collection in Myanmar. ZY, SM, SF, and MT performed the experiments. ZY, SM, and SF analyzed the data. SM, SK, and KO provided intellectual input, laboratory materials, reagents and analytic tools. YZ and SM wrote the manuscript. YZ, SM, KK, SB, SK, and KO revised the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgements
We thank all the farmers and veterinarians who kindly helped with sample collection. We thank Drs. Masayoshi Isezaki, Tomohiro Okagawa, and Naoya Maekawa, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, for their helpful advice. We would like to thank Editage (www.editage.jp) for English language editing. This research was supported in part by Grants-in-Aid for Scientific Research (B: 16H05804 and B: 18H02332) and a Young Scientists grant (B: 16K18798) from the Japan Society for the Promotion of Science.

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Figures
Figure 1

The phylogenetic trees based on the alignment of the Myanmar isolates and reference strains from four different gene fragments: (a) ICP4, (b) gB, (c) gG, and (d) gJ. The trees were generated using the neighbor-joining method coupled with the Kimura 2-parameter model and a bootstrap analysis of 1000 replicates.
