Molecular detection of infectious bronchitis and avian metapneumoviruses

in Oman backyard poultry

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**Abstract**

Infectious bronchitis virus (IBV) and avian metapneumovirus (aMPV) are economically important viral pathogens infecting chickens globally. Identification of endemic IBV and aMPV strains is central in controlling disease and production losses. Orophrayngeal swab samples were taken from 2317 birds within 243 different backyard flocks. Swabs from each flock were examined by RT-PCR using part-S1 and G gene primers for IBV and aMPV respectively. Thirty-nine flocks were positive for IBV and five flocks for aMPV. Five IBV genotypes were identified whereas all aMPV isolates belonged to subtype B. Data presented here demonstrates that both viruses, including recent variant IBVs, are endemic in Oman backyard poultry. Furthermore, 793/B-like IBV genotype was not limited to chicken flocks, but also found in one turkey and one duck flock. Though no disease was witnessed at the time of sampling, identified viruses may still pose a viable threat for both backyard and commercial poultry in Oman.

Keywords: infectious bronchitis virus; avian metapneumovirus; backyard poultry; surveillance; Oman

**Introduction**

Infectious bronchitis virus (IBV) is a highly contagious viral pathogen of chickens. It is a type 3 coronavirus and part of the family *Coronaviridae* ([Cavanagh, 2001](#_ENREF_9)). Most IBVs infect the respiratory, urinary and reproductive tracts causing considerable production losses ([Dolz et al., 2008](#_ENREF_14); [Jones, 2010](#_ENREF_20); [Roussan et al., 2008](#_ENREF_36); [Villarreal et al., 2007](#_ENREF_45); [Worthington et al., 2008](#_ENREF_46)). IBV infections can also be further aggravated by the presence of bacterial infections such as *Escherichia coli*, *Mycoplasma gallisepticum, Mycoplasma synoviae* and *Ornithobacterium rhinotracheale* ([Landman and Feberwee, 2004](#_ENREF_24); [Matthijs et al., 2003](#_ENREF_27); [Naqi et al., 2001](#_ENREF_32); [van Empel et al., 1996](#_ENREF_44)).

Since the first description of IBV in 1931 ([Schalk and Hawn, 1931](#_ENREF_37)), a number of different IBV genotypes have been detected worldwide ([Jackwood, 2012](#_ENREF_19)). Virulent IBV genotypes (eg. 793/B, QX, IS/1494/06, IS/885/00, Q1) that have a severe impact on chicken health and production have been reported in recent decades ([Gough et al., 1992](#_ENREF_17); [Kahya et al., 2013](#_ENREF_21); [Meir et al., 2004b](#_ENREF_30); [Yu et al., 2001](#_ENREF_47)). Infections from different IBV genotypes present a challenge for poultry producers worldwide ([Dolz et al., 2008](#_ENREF_14); [Jones, 2010](#_ENREF_20); [Worthington et al., 2008](#_ENREF_46)), and also for owners of backyard chicken flocks. The spike subunit 1 (S1) is highly variable in IBV and analysis of S1 using reverse transcription‑polymerase chain reaction (RT-PCR) and sequencing has allowed for genotyping of IBV strains ([Kingham et al., 2000](#_ENREF_22)) .

Avian metapneumovirus (aMPV) is an avian virus belonging to the *Paramyxoviridae* family ([Lee et al., 2007b](#_ENREF_26)). It is capable of infecting the respiratory tract of birds, causing avian rhinotracheitis in turkeys ([Jones, 2010](#_ENREF_20)) and swollen head syndrome in chickens ([Georgiades et al., 2001](#_ENREF_16)). Furthermore, it also causes a drop in egg production and/or egg quality in both turkeys and chickens ([Banet-Noach et al., 2005](#_ENREF_4); [Hess et al., 2004](#_ENREF_18)). The virus was first reported in South Africa in the 1970s ([Buys et al., 1989](#_ENREF_8)) and spread to other continents ([Jones, 2010](#_ENREF_20)). There are four distinct aMPV subtypes; A, B, C and D ([Cook and Cavanagh, 2002](#_ENREF_12)). Subtypes A and B are widespread throughout Asia, Europe, Africa and South America ([Jones, 2010](#_ENREF_20); [Kwon et al., 2010](#_ENREF_23); [Owoade et al., 2008](#_ENREF_34)). Reports of infections by subtypes C and D are infrequent and to date, subtype C has been reported in France, Korea and the US ([Alvarez et al., 2003](#_ENREF_2); [Bayon-Auboyer et al., 2000](#_ENREF_5); [Lee et al., 2007a](#_ENREF_25)), with D so far only being detected in France ([Bayon-Auboyer et al., 2000](#_ENREF_5)).

There is a particular paucity of information from Oman, with almost no published studies of avian respiratory viruses of any species. This is despite Oman’s geographic location, between the horn of Africa and southern Asia, and its importance as a site for migrating wild birds. Most poultry production in Oman is carried out in commercial farms; however there are around 25,000 backyard flocks bred for household consumption ([Oman, 2004](#_ENREF_33)). Maintaining a good health status of backyard flocks is crucial for both the flock owners, and the owners of nearby commercial flocks ([McBride et al., 1991](#_ENREF_28)). Backyard poultry in Oman are not vaccinated against IBV or aMPV.

This paper reports the first study on the prevalence of IBV genetic types and aMPV subtypes within backyard poultry in Oman.

**Materials and Methods**

**Sampling method.** Oropharyngeal swabs were collected from a total of 243 backyard flocks (2317 birds) from 237 farms within all regions and governorates of Oman (Figure 1), from June to September 2012. The samples were collected during a study on the prevalence of respiratory viruses, such as avian influenza (AI), Newcastle disease (ND), IB and aMPV. The number and location of sampled farms was determined based on the estimated prevalence of Avian Influenza (AI) and Newcastle Disease (ND) in Oman backyard poultry. Sampling criteria were calculated based on an estimated prevalence of AI of 30 % and between-cluster variance of 0.7. The number of flocks to be sampled was stratified by region according to the number of poultry farms, total number of poultry, number of people and number of backyard poultry present in each region. A confidence level of 95% was utilised along with a two- stage cluster sampling method ([Thrusfield, 1986](#_ENREF_43)).

The total backyard poultry population in Oman was estimated by the Ministry of Agriculture and Fisheries, Department of Rural Women Development to be approximately 10,000 poultry flocks with a median size of 50 birds per flock. The vast majority of the sampled farms raised local village chickens; however turkeys, guinea fowl, duck and geese were also present.

Local veterinarians and animal health engineers from the Ministry of Agriculture and Fisheries in each state aided with the selection of farms at different locations. Inclusion criteria involved a minimum distance between two farms (>1km) and <3 farms from each village. If only one flock was present at a farm, ten healthy adult (>3 months) birds were selected randomly and sampled. If more than one species of bird was present in the farm, then two flocks would be randomly chosen and ten birds sampled from each flock. If there were fewer than ten birds within a chosen flock, then all were sampled.

For detection of respiratory viruses, oropharyngeal swabs were collected from each flock and pooled into 1.5 ml distilled water in a sterile 5ml plastic bijou container. All samples were kept cool in crushed ice within a thermal-box and brought to the nearby Veterinary Research Centre. The bijou was vortexed and 100 µl was inoculated into the centre of a Flinders Technology Associates (FTA) card (Sigma Aldrich, Dorset, UK) using a sterile pipette and tips. Cards were left to dry for one hour at room temperature (22 ºC), away from direct light sources, then stored at 4 oC in air-tight plastic bags. Samples were transported to the University of Liverpool, UK, for processing and analysis.

Data such as sampling date, farm location (village, state, and region), species of birds, flock size, species of sampled birds, housing conditions, and water sources were recorded for each farm. Spatial coordinates of the location were recorded using GPS (Garmin GPS MAP 62s, USA).

**RNA extraction from FTA cards**. One circle from each FTA card was removed using sterile scissors and forceps and placed in a bijou containing 800-1000 µl of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), vortexed and incubated at room temperature for 10 min ([Abdelwhab et al., 2011](#_ENREF_1)). The supernatant was then used to extract viral RNA.

RNA extraction was performed using the QIAamp Viral RNA Mini Kit (Qiagen Ltd, Germany) according to the manufacturer’s instructions. Viral RNA was stored at -20 oC until required.

**aMPV RT-PCR.** RT-PCR was performed on each of the 243 RNA extracts using both primers and cycle conditions as previously published ([Cavanagh et al., 1999](#_ENREF_10)). A 268 bp band corresponds to type A, whereas a 361 bp band is type B.

Positive isolates were typed by comparison of amplicon size to an aMPV type B positive control.

**IBV RT-PCR and amplicon sequencing.** All samples were subjected to RT-PCR to detect IBV positive flocks. Primers and cycle conditions were as previously described ([Cavanagh et al., 1999](#_ENREF_10)). The presence of a 380 bp amplicon (relating to the S1 gene) demonstrates that the sample is positive for IBV. The IBV M41 strain was utilised as the positive control.

Positive PCR reactions were purified using 0.15 µl exonuclease 1 (EXO) and 0.99 µl of shrimp alkaline phosphatase (SAP). The mixture was incubated at 37 oC for 30 minutes and then a further 10 minutes at 80 oC to remove any residual impurities. Purified DNA along with the forward primer SX3+ was sent for commercial sequencing (Source Bioscience Ltd, Nottingham, UK).

**Phylogenetic analysis of sequenced isolates.** Sequences were initially analysed in ChromasPRO v1.7.3 (http://technelysium.com.au/) to confirm good quality read data. Alignments were carried out in MEGA6 ([Tamura et al., 2013](#_ENREF_41)) using Clustal W ([Thompson et al., 1994](#_ENREF_42)). Following alignment, BLAST searches were conducted to confirm isolate identification. Obtained IBV sequences were compared against reference strains for S1 retrieved from GenBank (National Centre of Biotechnology Information). Reference strains used throughout this study were UK/3/91 (Z83977), UK/4/91 (JN600614), 793/B (Z83979), CR88121 (JN542567), IS/1494/06 (EU780077), IS/885/00 (AY279533), Eg/1212B (JQ839287), Q1 (AF286302), QX (AF193423), M41 (GQ219712) and D274 (X15832).

Maximum likelihood analysis was utilised to infer evolutionary trees of both isolate and reference sequences, with default settings and 1000 bootstrap re-sampling. BLAST was utilised for nucleotide and amino acid identity comparisons between representative isolates within identified clusters.

**Results**

**Detection of aMPV.** From the total of 243 flocks sampled during the study, five tested aMPV positive (2.06%). All five samples were from chickens and all were identified as aMPV subtype B. The positive samples came from northern regions of Oman; three from the Ad Dhahirah region (Albrimi, Ibri and Dank), one from the Ash Sharqiyah region (Snaw) and the fifth from the Muscat Governorate (Qurayat). Four of the infected flocks were located within the mainland, with a single flock from the coastal town of Qurayat (Figure 2A).

**Detection and genotyping of IBV.** From the 243 flocks assayed using RT-PCR, a total of 39 tested positive for IBV (16.05%) (Figure 2B). Of the 39 isolates sequenced, it was possible to determine the genotypes of 32 (82.05%) using BLAST (Table 2). A total of five genotypes were represented within the sample size. The majority of samples showed greatest homology to genotype 793/B (n=26/39; 66.67%), with the remaining isolates relating closely to M41 (n=2/39; 5.12%), D274 (n=2/39; 5.12%), IS/1494/06 (n=1/39; 2.56%) and IS/885/00 (n=1/39; 2.56%). All 32 sequenced samples were submitted to GenBank and assigned accession numbers (Figure 3).

The Al Wusta region demonstrated the highest flock prevalence rate (37.5%) followed by the Dhofar Governorate (28.5%). The 793/B-like genotype was identified from all regions and governorates, except the Muscat Governorate which remained the only region to have no IBV positive flocks. Dhofar Governorate was the only infected region to demonstrate a single genotype (793/B), while the other infected regions had a presence of ≥2 genotypes. All IBV infected flocks consisted of solely chickens, with the exception of one duck and one turkey flock.

Genotype 793/B was detected in the duck and turkey flocks from Ad Dakhliyah and Al Batinah regions respectively. The 793/B genotype was also detected within chicken flocks from these regions.

**Phylogenetic analysis of IBV isolates.** Results from the maximum likelihood analysis demonstrate that the isolates formed five distinct clusters (Figure 3), relating to genotypes of strains previously reported. Representative isolates from each of the five clusters were chosen for comparison of nucleotide and amino acid similarities (Table 3).

The majority of the 26 isolates clustering with UK/4/91 had between 96-100% nucleotide homology (resulting in 92-100% amino acid similarity). Sample Oman-Lewa-4-12 (Al Batinah) had the lowest homology however still clustered with 793/B. The IS/885/00‑like strain had a higher variation from the first isolate with 90% nucleotide homology. The three other genotypes demonstrated minimal nucleotide variation from the reference strains: IS/1494/06‑like had 99% homology, M41-like had 99% homology and D274-like had 98% homology.

**Discussion**

We present the first study to identify the circulating IBV genotypes and aMPV subtypes in backyard flocks in Oman. For this study, an epidemiologically representative number of samples were collected from different regions of Oman and transferred to the University of Liverpool on FTA cards. The cards inactivate genomic material and allow for RNA extraction once back in the laboratory, proving useful for analysing field isolates away from the point of sampling. The successful use of the cards for both IBV and aMPV has been previously described ([Awad et al., 2014](#_ENREF_3); [Moscoso et al., 2005](#_ENREF_31)). Using RT-PCR and direct amplicon sequencing, we identified the prevalence of both viruses within backyard flocks in relation to location and poultry species.

Thirty-nine flocks were identified as IBV positive, with an overall prevalence of 16.04%. This finding is lower than previously reported in production farms within neighbouring countries, for example 58.8% and 42.8% in Jordan and Iran respectively ([Roussan et al., 2009](#_ENREF_35); [Seyfi Abad Shapouri et al., 2004](#_ENREF_39)). However both studies sampled chickens within a higher density environment, which may have contributed to the higher prevalence rate compared to the backyard flocks.

In this study, 793/B was the dominant IBV genotype infecting backyard flocks, with an overall prevalence of 66.67% in IBV-positive flocks. The presence of 793/B in commercial flocks has previously been reported in other Middle East countries such as Iran, Jordan and Israel ([Meir et al., 2004b](#_ENREF_30); [Roussan et al., 2008](#_ENREF_36); [Seyfi Abad Shapouri et al., 2004](#_ENREF_39)). In the last few years 793/B vaccines, and variants closely-related to 793/B, have been detected in the Omani commercial chicken farms (K Ganapathy, unpublished data). The 793/B isolates detected within the backyard flocks formed a close phylogenetic cluster, indicating that potentially the same 793/B strain may be circulating between different Omani backyard flocks and regions.

The Mass serotype was the first to be isolated in the 1930’s ([Jackwood, 2012](#_ENREF_19)) and adopted for use in early IBV vaccines ([Jackwood, 2012](#_ENREF_19); [Sjaak de Wit et al., 2011](#_ENREF_40)). Strains belonging to the D274 serotype were first isolated in the Netherlands ([Davelaar et al., 1984](#_ENREF_13)) and later developed as a vaccine. In the current study, we detected M41-like and D274-like infections within four chicken flocks at a low prevalence of 0.82% each. Both genotypes have been previously reported in commercial flocks in the Middle East ([Roussan et al., 2008](#_ENREF_36); [Seyfi Abad Shapouri et al., 2004](#_ENREF_39); [Sjaak de Wit et al., 2011](#_ENREF_40)), albeit at a much higher prevalence than witnessed during this study. Despite no clinical disease being identified in the M41 or D274 positive birds, both viruses are known for their ability to cause disease in chickens ([Bourogaa et al., 2009](#_ENREF_6); [Feng et al., 2012](#_ENREF_15)).

IBV genotypes IS/1494/06 and IS/885/00 were originally isolated in Israel and are currently circulating in a number of Middle East countries ([Kahya et al., 2013](#_ENREF_21); [Meir et al., 2004a](#_ENREF_29)). Here, for the first time, we report the detection of these important Middle East IBV variants in backyard flocks. Even though a low prevalence was witnessed in this study, their importance cannot be ignored due to their ability to cause severe respiratory, reproductive and renal diseases ([Kahya et al., 2013](#_ENREF_21)). There variations pose a threat not only to backyard flocks but also to the commercial poultry industry in Oman and possibly further afield in the region.

It is of interest to note that IBV genotype 793/B was detected in a flock of Muscovy ducks from the Ad Dakhliyah region and in a turkey flock in the Al Batinah region. This presence suggests that common circulating IBV genotypes (such as 793/B) could potentially establish an infection in both ducks and turkeys. Previous reports of IBV in apparently healthy ducks have been reported from China and Nigeria using RT-PCR ([Feng et al., 2012](#_ENREF_15); [Semeka et al., 2013](#_ENREF_38)). Despite previous reports having identified a turkey coronavirus isolate closely related to IBV ([Breslin et al., 1999](#_ENREF_7); [Cavanagh et al., 2001](#_ENREF_11)), to date there has been only one previous report of IBV infecting turkeys ([Semeka et al., 2013](#_ENREF_38)). To our knowledge, this appears to be the first report of an IBV-like detection in both duck and turkey flocks in the Middle East.

Five of the 243 sampled flocks were aMPV positive. All aMPV positive samples from this study were of subtype B and were isolated from five different states within four northern regions, highlighting the sporadic distribution pattern of aMPV in the backyard flocks. Although subtype A was not identified in this study, co‑circulation of both subtypes A and B within commercial flocks in the Middle East has been previously reported ([Banet-Noach et al., 2005](#_ENREF_4)). This appears to be the first detection of aMPV within backyard flocks in Oman. With its involvement in respiratory and reproductive disorders ([Georgiades et al., 2001](#_ENREF_16); [Jones, 2010](#_ENREF_20)), the existence of this pathogen is likely to pose a threat to backyard and commercial poultry in Oman.

Our study demonstrates the complex epidemiology of both IBV and aMPV in backyard flocks in Oman. The reasons for the predominant detection of 793/B-like over other IBV strains and the presence of a single aMPV subtype (subtype B) are unknown. Further to this, the potential sources of these viruses in the sampled flocks are not known. The detection of 793/B in duck and turkey flocks highlights the possible role of these birds as potential mechanical or biological carriers. With the expanding commercial poultry industry and the increasing role of backyard poultry in Oman, it is essential to improve our understanding on the epidemiology of IBV and aMPV for better control of these pathogens.

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**Table 1:** The number of flocks, and birds of each poultry species, sampled in eight regions of Oman.

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Region** | **Total number of birds** | **Number of flocks** | **Hens** | **Turkeys** | **Ducks** | **Geese** | **Guinea fowls** |
| AL BATINAH REGION | 787 | 81 | 669 | 15 | 88 | 5 | 10 |
| ADH DHAHIRAH REGION | 462 | 47 | 452 | 10 | 0 | 0 | 0 |
| MUSCAT GOVERNORATE | 130 | 13 | 111 | 9 | 10 | 0 | 0 |
| AD DAKHLIYAH REGION | 363 | 41 | 333 | 0 | 30 | 0 | 0 |
| ASH SHARQIYAH REGION | 345 | 35 | 315 | 10 | 10 | 0 | 10 |
| DHOFAR GOVERNORATE | 70 | 7 | 70 | 0 | 0 | 0 | 0 |
| AL WUSTA REGION | 76 | 8 | 76 | 0 | 0 | 0 | 0 |
| MUSANDAM GOVERNORATE | 84 | 11 | 80 | 0 | 4 | 0 | 0 |
| **Total** | **2317** | **243** | **2106** | **44** | **142** | **5** | **20** |

**Table 2:** Prevalence of identified genotypes within each sampled region from 39 IBV positive isolates.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Region** | **Total number of flocks** | **Total number of flocks with IBV+ isolations** | **Number of IBV positive isolates** | | | | | **Non-interpretable isolates1** |
| *793/B-like* | *M41-like* | *D274-like* | *IS/885-like* | *IS/1494-like* |
| AL BATINAH REGION | 81 | 12 (14.81%) | 9 | - | - | - | - | 3 |
| ADH DHAHIRAH REGION | 47 | 7 (14.89%) | 2 | - | 1 | - | 1 | 3 |
| MUSCAT GOVERNORATE | 13 | 0 | - | - | - | - | - | 0 |
| AD DAKHLIYAH REGION | 41 | 9 (21.95%) | 7 | 1 | - | - | - | 1 |
| ASH SHARQIYAH REGION | 35 | 5 (14.29%) | 3 | 1 | 1 | - | - | 0 |
| DHOFAR GOVERNORATE | 7 | 2 (28.57%) | 2 | - | - | - | - | 0 |
| AL WUSTA REGION | 8 | 3 (37.5%) | 2 | - | - | 1 | - | 0 |
| MUSANDAM GOVERNORATE | 11 | 1 (9.09%) | 1 | - | - | - | - | 0 |
| ***TOTAL*** | *243* | *39* | *26* | *2* | *2* | *1* | *1* | 7 |
| OVERALL PREVALENCE OF IBV GENOTYPE (%) |  | 16.05 | 10.70 | 0.82 | 0.82 | 0.41 | 0.41 |  |
| NUCLEOTIDE IDENTITY RANGE OF ISOLATES COMPARED TO GENOTYPE (%) |  |  | 91-98 | 99-100 | 98-99 | 90 | 99 |  |

1Isolate was sequenced but either failed or returned poor sequence data

**Table 3:** Nucleotide and amino acid similarity between representatives from each distinct cluster (n=9) and closely related reference strains (n=7) for partial S1 sequences

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Nucleotide Identity (%)** | | | | | | | | | | | | | | | | | | | | |
|  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |  |  |  |  |
| **1** |  | 82 | 81 | 82 | 82 | 82 | 81 | 82 | 82 | 98 | 82 | 81 | 83 | 79 | 83 | 83 | **1** | **Oman/Snaw/6/12** | |  |
| **2** | 62 |  | 81 | 81 | 78 | 79 | 78 | 78 | 79 | 82 | 99 | 81 | 82 | 81 | 79 | 77 | **2** | **Oman/Manah/2/12** | | |
| **3** | 63 | 61 |  | 89 | 82 | 81 | 80 | 81 | 80 | 81 | 81 | 99 | 89 | 90 | 81 | 80 | **3** | **Oman/Mosira/5/12** | | |
| **4** | 68 | 75 | 76 |  | 83 | 81 | 80 | 80 | 80 | 82 | 80 | 89 | 99 | 90 | 82 | 80 | **4** | **Oman/Ibri/4/12** | |  |
| **5** | 61 | 64 | 68 | 70 |  | 97 | 96 | 97 | 97 | 82 | 78 | 83 | 84 | 83 | 97 | 95 | **5** | **Oman/Mohot/1/12** | | |
| **6** | 62 | 66 | 64 | 66 | 94 |  | 99 | 99 | 99 | 82 | 78 | 81 | 82 | 81 | 98 | 96 | **6** | **Oman/Galan/1/12** | | |
| **7** | 60 | 63 | 62 | 63 | 92 | 98 |  | 99 | 99 | 81 | 78 | 80 | 81 | 80 | 98 | 96 | **7** | **Oman/Alhamra/4/12** | | |
| **8** | 60 | 65 | 64 | 64 | 93 | 99 | 99 |  | 100 | 82 | 79 | 81 | 81 | 81 | 98 | 96 | **8** | **Oman/Ibra/4/12** | |  |
| **9** | 60 | 65 | 63 | 64 | 93 | 99 | 99 | 100 |  | 82 | 78 | 81 | 81 | 80 | 98 | 96 | **9** | **Oman/Bidbid/1/12** | | |
| **10** | 95 | 62 | 59 | 67 | 61 | 62 | 60 | 62 | 61 |  | 82 | 82 | 82 | 80 | 84 | 84 | **10** | **D274** | |  |
| **11** | 62 | 100 | 60 | 75 | 53 | 54 | 52 | 54 | 53 | 63 |  | 82 | 81 | 81 | 79 | 78 | **11** | **M41** | |  |
| **12** | 64 | 61 | 98 | 76 | 61 | 60 | 58 | 60 | 59 | 62 | 61 |  | 89 | 90 | 81 | 80 | **12** | **Eg/1212B** | |  |
| **13** | 68 | 60 | 76 | 96 | 65 | 62 | 60 | 62 | 61 | 60 | 60 | 76 |  | 91 | 83 | 81 | **13** | **IS/1494/06** | |  |
| **14** | 61 | 62 | 76 | 77 | 63 | 59 | 57 | 58 | 57 | 58 | 62 | 76 | 79 |  | 82 | 80 | **14** | **IS/885/00** | |  |
| **15** | 62 | 66 | 64 | 64 | 94 | 98 | 96 | 96 | 97 | 63 | 66 | 59 | 63 | 62 |  | 98 | **15** | **UK/4/91** | |  |
| **16** | 62 | 64 | 61 | 60 | 89 | 93 | 92 | 92 | 93 | 63 | 64 | 58 | 59 | 58 | 96 |  | **16** | **CR88121** | |  |
|  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |  |  |  |  |
| **Amino Acid Identity (%)** | | | | | | | | | | | | | | | | | | | | |

**Figure 1:** Distribution of sampled farms from each region within Oman. 1 = Ad Dakhliyah, 2 = Adh Dhahirah, 3 = Al Batinah, 4 = Al Wusta, 5 = Ash Sharqiyah, 6 = Dhofar Governorate, 7 = Musandam Governorate, 8 = Muscat Governorate

**Figure 2:** **(A)** Locations of aMPV positive flocks (n=5). **(B)** Locations of IBV positive flocks (n=39). 1 = Albrimi, 2 = Ibri, 3 = Dank, 4 = Snaw and 5 = Qurayat.

**Figure 3:** Maximum likelihood analysis of 32 isolates (accession numbers included in brackets) obtained from regions of Oman, and 11 IBV reference strains from GenBank.