**Supplemental files**

**Table S1:** Epidemiological details for each sampled holding collected from backyard ducks in Bangladesh

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Collection Date** | **Sampling Area** | **Total number of samples in the area** | **Sample Name** | **Number of samples** | **Number of birds per household** | **Age** | **Breeds** | **Clinical status** | **Other poultry in the household** | **Distance to 1LBM** |
| 06.12.18 | Mymensingh  City | 16 | SAD-A1 to SAD-A11 | 11 | 11 | 6 Months | Khaki Campbell, Nageswari | 2AH | Chicken | 2 Km |
| SAD-B1 to SAD-B5 | 5 | 5 | 7 Months | Khaki Campbell, Nageswari | AH | Chicken | 2 Km |
| 07.12.18 | Kalibazail  Fulbaria,  Mymensingh | 166 | Ful-A1 to Ful-A4 | 4 | 4 | 7 Months | Khaki Campbell | AH | Chicken | 0.5 Km |
| Ful-B1 to Ful-B4 | 4 | 6 | 7 Months | Khaki Campbell | 3AH and 30% MPF | Chicken | 0.5 Km |
| Ful-C1 to Ful-C5 | 5 | 5 | 7 Months | Khaki Campbell,,Indian runner | AH | Chicken, Pigeon | 0.5 Km |
| Ful-D1 to Ful-D4 | 4 | 5 | 8 Months | Khaki Campbell | AH | Chicken | 0.5 Km |
| Ful-E1 to Ful-E9 | 9 | 9 | 7 Months | Rouen | AH | Chicken, Pigeon, Turkey | 0.5 Km |
| Ful-F1 to Ful-F7 | 7 | 7 | 7 Months | Khaki Campbell (Anas platyrhynchos domesticus) | AH | Chicken, Turkey | 0.5 Km |
| Ful-G1 to Ful-G6 | 6 | 6 | 3 Years | Khaki Campbell | AH | Chicken, Pigeon, Turkey | 0.5 Km |
| Ful-H1 to Ful-H5 | 5 | 5 | 2 Years | Khaki Campbell | AH and 20% MPF | Chicken | 0.5 Km |
| Ful-I1 to Ful-I2 | 2 | 4 | 1 Year | Khaki Campbell | AH | Chicken | 0.5 Km |
| Ful-J1 to Ful-J4 | 4 | 4 | 1 Year | Khaki Campbell | AH | Chicken | 0.5 Km |
| Ful-K1 to Ful-K3 | 3 | 4 | 1 Year | Khaki Campbell | AH | Chicken, Pigeon, Guineafowl | 0.5 Km |
| Ful-L1 to Ful-L3 | 3 | 5 | 1 Year | Khaki Campbell | AH | Chicken, Pigeon, Guineafowl | 0.5 Km |
| Ful-M1 to Ful-M2 | 2 | 5 | 7 Months | Khaki Campbell | AH | Chicken | 0.5 Km |
| Ful-N1 to Ful-N6 | 6 | 7 | 7 Months | Khaki Campbell | AH | Chicken, Pigeon | 0.5 Km |
| Chorkalibaza,  Fulbaria,  Mymensingh | Ful-O1 to Ful-O4 | 4 | 7 | 1 Year 4 Months | Khaki Campbell,Indian runner | AH | Chicken | 0.5 Km |
| Ful-P1 to Ful-P2 | 2 | 5 | 1.5 Years | Khaki Campbell, Pekin | AH and 50% MPF | Chicken | 0.5 Km |
| Ful-Q1 to Ful-Q4 | 4 | 7 | 1 Year | Khaki Campbell | AH | No | 0.5 Km |
| Ful-R1 to Ful-R4 | 4 | 5 | 2.5 Years | Khaki Campbell, Pekin | AH | Chicken | 0.5 Km |
| Ful-S1 to Ful-S8 | 8 | 8 | 3 Years | Khaki Campbell | AH | Chicken | 0.5 Km |
| Ful-T1 to Ful-T13 | 13 | 16 | 6 Months | Khaki Campbell | AH | Chicken | 0.5 Km |
| Ful-U1 to Ful-U2 | 2 | 2 | 2 Years | Khaki Campbell | AH | Chicken | 0.5 Km |
| Ful-V1 | 1 | 4 | 7 Months | Khaki Campbell | AH | Chicken | 0.5 Km |
| Ful-W1 to Ful-W6 | 6 | 6 | 1.5 Years | Khaki Campbell | AH | Chicken | 0.5 Km |
| Kalibazail,  Fulbaria  Mymensingh | Ful-X1 to Ful-X12 | 12 | 12 | 1.5 Years | Khaki Campbell | AH | Chicken, Pigeon | 2 Km |
| Ful-Y1 to Ful-Y2 | 2 | 2 | 1 Year | Khaki Campbell | AH | Chicken, Pigeon | 2 Km |
| Ful-Z1 to Ful-Z7 | 7 | 7 | 7 Months | Khaki Campbell | AH | Chicken, Pigeon | 2 Km |
| Ful-AA1 to Ful-AA9 | 9 | 11 | 1.5 Years | Khaki Campbell | AH | Chicken | 2 Km |
| Ful-BB1 to Ful-BB11 | 11 | 11 | 8 Months | Khaki Campbell | AH and 75% MPF | Chicken | 2 Km |
| Ful-CC1 to Ful-CC9 | 9 | 11 | 1 Year | Khaki Campbell | AH | Chicken | 2 Km |
| Ful-DD1 to Ful-DD8 | 8 | 8 | 1 Year | Khaki Campbell | AH | Chicken | 2 Km |
| 08.12.18 | Bibirchor, Talki, Nakla, Sherpur | 149 | Nak-1a to Nak-1g | 7 | 7 | 7 Months | Khaki Campbell, Pekin, Indian runner | AH | Chicken | 2 Km |
| Nak-2a to Nak- 2h | 8 | 12 | 8 Months | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-3a to Nak- 3f | 6 | 10 | 8 Months | Khaki Campbell, Pekin, Indian runner | AH | Chicken | 2 Km |
| Nak-4a to Nak- 4g | 7 | 13 | 6 Months | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-5a to Nak- 5b | 2 | 11 | 7 Months | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-6a to Nak- 6c | 3 | 8 | 1 Year | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-7a to Nak- 7l | 12 | 15 | 2 Years | Khaki Campbell, Pekin, Indian runner | AH | No | 2 Km |
| Nak-8a to Nak- 8l | 12 | 12 | 6 Months | Khaki Campbell, Pekin, Indian runner | AH | Chicken | 2 Km |
| Nak-9a to Nak- 9k | 11 | 12 | 6 Months | Khaki Campbell, Pekin, Indian runner | AH and 40% MPF | Chicken | 2 Km |
| Nak-10a to Nak- 10i | 9 | 10 | 7 Months | Khaki Campbell, Pekin, Indian runner | AH | Chicken, Dove | 2 Km |
| Nak-11a to Nak- 11e | 5 | 9 | 8 Months | Khaki Campbell, Pekin, Indian runner | AH | Chicken | 2 Km |
| Nak-12a to Nak- 12b | 2 | 4 | 8 Months | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-13a to Nak- 13j | 10 | 10 | 1 Year | Khaki Campbell | AH | Chicken, Pigeon | 2 Km |
| Nak-14a to Nak- 14c | 3 | 7 | 8 Months | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-15a to Nak- 15d | 4 | 5 | 8 Months | Khaki Campbell, Pekin, Indian runner | AH | Chicken | 2 Km |
| Nak-16a to Nak- 16b | 2 | 2 | 1 Year | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-17a to Nak- 17c | 3 | 3 | 6 Months | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-18a to Nak- 18h | 8 | 12 | 7 Months | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-19a to Nak- 19b | 2 | 2 | 8 Months | Khaki Campbell, Pekin, Indian runner | AH | Chicken | 2 Km |
| Nak-20a to Nak- 20d | 4 | 10 | 7 Months | Khaki Campbell, Pekin, Indian runner | AH | Chicken, Pigeon | 2 Km |
| Nak-21a to Nak- 21e | 5 | 9 | 7 Months | Khaki Campbell | AH | Chicken, Pigeon | 2 Km |
| Nak-22a to Nak- 22d | 4 | 6 | 8 Months | Khaki Campbell | AH | Chicken, Pigeon, Mynah | 2 Km |
| Nak-23a to Nak- 23c | 3 | 3 | 8 Months | Khaki Campbell | AH | Chicken, Pigeon | 2 Km |
| Nak-24a to Nak- 24e | 5 | 9 | 1 Year | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-25a to Nak- 25d | 4 | 4 | 6 Months | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-26a to Nak- 26c | 3 | 10 | 1 Year | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-27a to Nak- 27c | 3 | 3 | 8 Months | Khaki Campbell | AH | Chicken | 2 Km |
| Nak-28a to Nak- 28b | 2 | 5 | 8 Months | Khaki Campbell | AH | Chicken | 2 Km |
| 10.12.18 | Pouroshova, Signboard, Madan, Netrokona | 74 | Mad-1a to Mad-1e | 5 | 6 | 1 Year | Khaki Campbell | AH and 50% MPF | Pigeon | 0.5 Km |
| Mad-2a to Mad-2j | 10 | 10 | 6 Months | Khaki Campbell, Pekin, Indian runner | AH | Chicken | 0.5 Km |
| Mad-3a to Mad-3j | 10 | 10 | 6 Months | Khaki Campbell, Pekin, Indian runner | AH | Chicken | 0.5 Km |
| Mad-4a to Mad-4f | 6 | 11 | 1 Year | Khaki Campbell, Pekin, Indian runner | AH and 50% MPF | Chicken, Pigeon | 0.5 Km |
| Mad-5a to Mad-5e | 5 | 11 | 1 Year | Khaki Campbell, Pekin, Indian runner | AH and 50% MPF | Chicken, Pigeon | 0.5 Km |
| Mad-6a to Mad-6c | 3 | 3 | 1 Year | Khaki Campbell | AH and 28% MPF | Chicken | 3 Km |
| Mad-7a to Mad-7d | 4 | 5 | 1 Year | Khaki Campbell | AH | Chicken | 3 Km |
| Mad-8a to Mad-8c | 3 | 3 | 1 Year | Khaki Campbell | AH | Chicken | 3 Km |
| Mad-9a | 1 | 2 | 1 Year | Khaki Campbell | AH | Chicken | 3 Km |
| Mad-10a to Mad-10d | 4 | 4 | 8 Months | Khaki Campbell, Pekin, Indian runner | AH and 60% MPF | Chicken, Pigeon | 3 Km |
| Mad-11a to Mad-11c | 3 | 3 | 1 Year | Khaki Campbell | AH | Pigeon | 3 Km |
| Mad-12a to Mad-12d | 4 | 4 | 1 Year | Khaki Campbell, Pekin | AH | Pigeon | 3 Km |
| Mad-13a to Mad-13f | 6 | 6 | 1 Year | Khaki Campbell, Pekin, Indian runner | AH and 15% MPF | Chicken, Pigeon | 3 Km |
| Mad-14a to Mad-14e | 5 | 10 | 1 Year | Khaki Campbell, Pekin | AH | Chicken | 200m |
| Mad-15a to Mad-15e | 5 | 10 | 1 Year | Khaki Campbell, Pekin | AH | Chicken | 200m |
| 09.01.19 | kishoreganj  City | 95 | Kis-1a to 1j | 10 | 15 | 6 Months | Khaki Campbell | AH | No | 1.5Km |
| Kis-2a to 2g | 7 | 10 | 1 Year | Khaki Campbell | AH | No | 1.5Km |
| Kis-3a to 3f | 6 | 11 | 1 Year | Khaki Campbell | AH | Chicken | 1.5Km |
| Kis-4a to 4e | 5 | 7 | 1 Year | Khaki Campbell | AH | Chicken | 1.5Km |
| Kis-5a to 5c | 3 | 8 | 6 Months | Khaki Campbell | AH | No | 1.5Km |
| Kis-6a to 6e | 5 | 12 | 6 Months | Khaki Campbell | AH | Chicken | 1.5Km |
| Kis-7a to 7c | 3 | 7 | 1 Year | Khaki Campbell | AH | Chicken | 1.5Km |
| Kis-8a to 8e | 5 | 9 | 1 Year | Khaki Campbell | AH | Chicken | 1.5Km |
| Kis-9a to 9f | 6 | 10 | 1 Year | Khaki Campbell, Pekin, Indian runner | AH | Chicken | 1.5Km |
| Kis-10a to 10e | 5 | 9 | 1 Year | Khaki Campbell | AH | Chicken, Pigeon | 1.5Km |
| Kis-11a to 11b | 2 | 6 | 1 Year | Khaki Campbell | AH | Chicken, Pigeon | 1.5Km |
| Kis-12a to 12d | 4 | 7 | 3 Years | Khaki Campbell | AH | Chicken | 1.5Km |
| Kis-13a to 13c | 3 | 7 | 2 Years | Khaki Campbell, Pekin | AH | Chicken, Pigeon | 1.5Km |
| Kis-14a to 14d | 4 | 8 | 1 Year | Khaki Campbell | AH | Chicken | 1.5Km |
| Kis-15a to 15c | 3 | 15 | 3 Months | Khaki Campbell | AH | No | 1.5Km |
| Kis-16a to 16d | 4 | 20 | 1 Year | Khaki Campbell | AH | Chicken | 1.5Km |
| Kis-17a to 17c | 3 | 21 | 1 Year | Khaki Campbell | AH | No | 1.5Km |
| Kis-18a to 18d | 4 | 9 | 1 Year | Khaki Campbell, Pekin | AH | Chicken | 1.5Km |
| Kis-19a to 19c | 3 | 8 | 1 Year | Khaki Campbell | AH | Chicken | 1.5Km |
| Kis-20a to 20d | 4 | 10 | 1 Year | Khaki Campbell | AH | No | 1.5Km |
| Kis-21a to 21c | 3 | 12 | 1 Year | Khaki Campbell | AH | Chicken | 1.5Km |
| Kis-22a to 22c | 3 | 5 | 3 Years | Khaki Campbell | AH | Chicken | 1.5Km |

1 Live bird market

2Apparently healthy

3 Percent mortality in previous flock

**Table S2:** List of H4 primers used to amplify full length HA gene segment

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence** | **Position** |
| H4\_F1 | GGA AGT GGT CAC TGC ACA | 157-174 |
| H4\_F2 | ACA ATG GGA AAT TCG AAT TCA TTG | 396-419 |
| H4\_F3 | CAT AGA GAA TGG ATG GCA AG | 1075-1094 |
| H4\_R1 | TAC AAA TGG ATA GCA RGT GTC | 340-321 |
| H4\_R2 | CAA TGA ATT CRA ATT TCC CAT TG | 419-397 |
| H4\_R3 | CAG AAT TGT GCT CTT CTT CTG | 864-845 |
| H4\_R4 | TGC AAA TCT GGC ACC GGA T | 1709-1691 |

**Table S3:** PCR reactions and thermal profile standardized in this study for the detection of avian viruses

|  |  |  |
| --- | --- | --- |
| **Viruses** | **Reaction mixture\*** | **Thermal profile** |
| Avian paramyxovirus 1 | RNase free water: 0.9µl  2x RT-PCR Buffer: 6.0µl  RT-PCR Enzyme Mix: 0.5µl  Primer 1066 FW\_20 pmol: 0.5µl  Primer 1231 Rev\_20 pmol: 0.5µl  APMV-1-Mix-FAM: 1.6µl  RNA template: 2.5µl | RT: 10 min 45°C  Taq activation: 10 min 95°C  45 cycles of  Denaturation: 15 sec 95°C  Annealing: 30 sec 55°C  Elongation: 30 sec 72°C |
| Anatid herpes virus 1 | RNase free water: 3µl  2x RT-PCR Buffer: 5.0µl  AHV-1-Mix-FAM: 2µl  DNA template: 2.5µl | Taq activation: 10 min 95°C  45 cycles of  Denaturation: 15 sec 95°C  Annealing: 1 min 53°C  Extension: 30sec 72°C |
| Avian borna virus | RNase free water: 1µl  2x RT-PCR Buffer: 5.0µl  RT-PCR Enzyme Mix: 0.5µl  ABBV-1-Mix-FAM: 3.5µl  RNA template: 2.5µl | RT: 10 min 45°C  Taq activation: 10 min 95°C  45 cycles of  Denaturation: 15 sec 95°C  Annealing: 30 sec 55°C  Elongation: 30 sec 72°C |
| Avian parvovirus | RNase free water: 2.5µl  2x RT-PCR Buffer: 5.0µl  RT-PCR Enzyme Mix: 0.5µl  APV-1-Mix-FAM: 2 µl  DNA template: 2.5µl | Taq activation 10 min 95°C  45 cycles of  Denaturation: 15 sec 95°C  Annealing: 30sec 53°C  Extension: 30sec 72°C |

\* All reaction mixture prepared following the AgPath-ID One-Step kit (Applied Biosystems, Foster City, CA)

**Table S4**: Sequences generated in this study with their accession numbers

|  |  |  |
| --- | --- | --- |
| **Strains** | **Genes** | **Accession numbers** |
| A/duck/Bangladesh/Nak6b/2019 | AIV H4 and N6 | EPI1649018 and EPI1649019 |
| A/duck/Bangladesh/Nak7i/2019 | - | EPI1649020 and EPI1649021 |
| A/duck/Bangladesh/Nak7j/2019 | - | EPI1649022 and EPI1649023 |
| AvCoV\_duck\_Bangladesh\_SadA9\_2019 | AvCoV Polymerase | MN968769 |
| AvCoV\_duck\_Bangladesh\_Nak1c \_2019 | - | MN968770 |
| AvCoV\_duck\_Bangladesh\_Kis20\_2019 | - | MN968771 |

* Same in line above