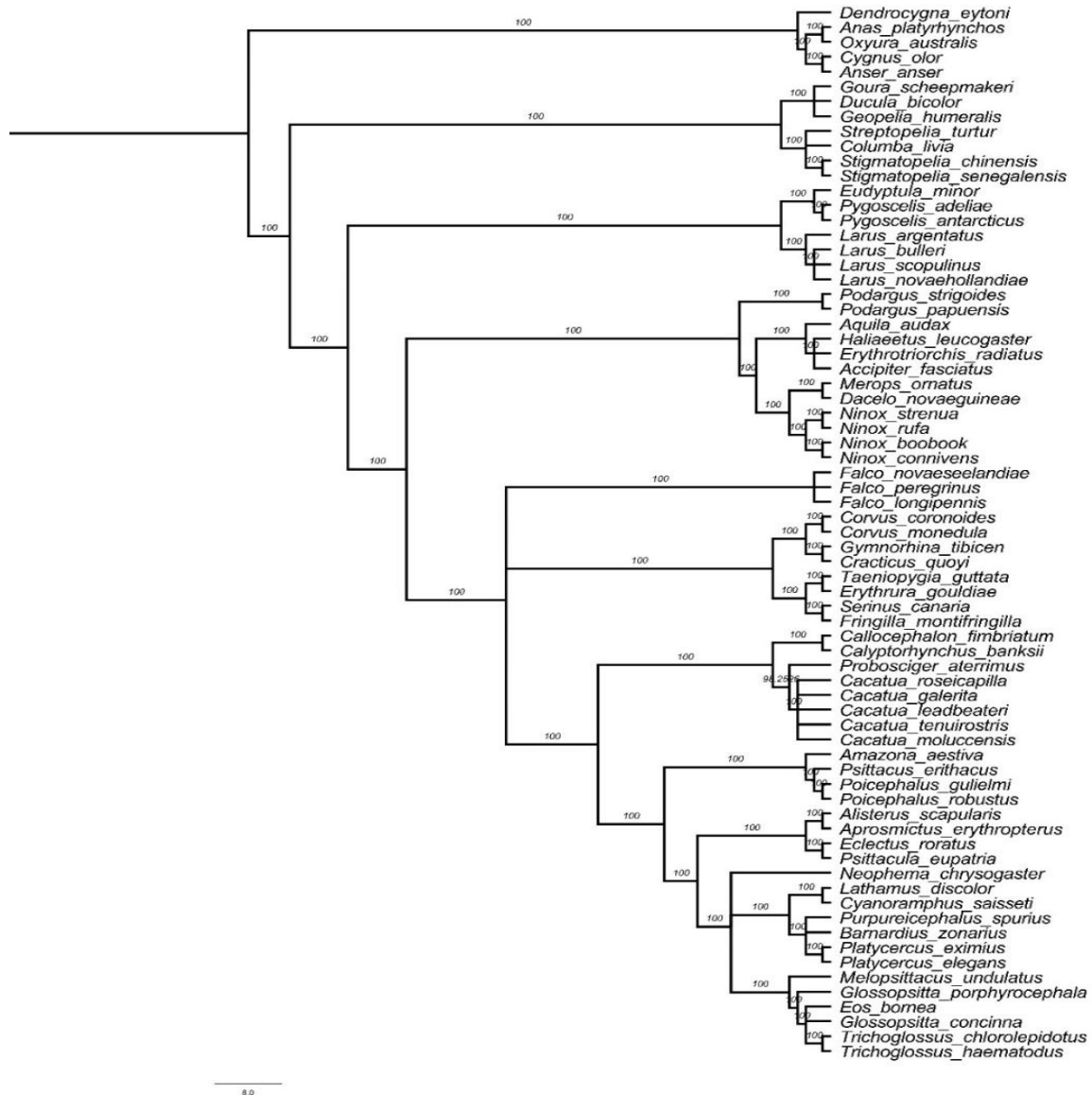


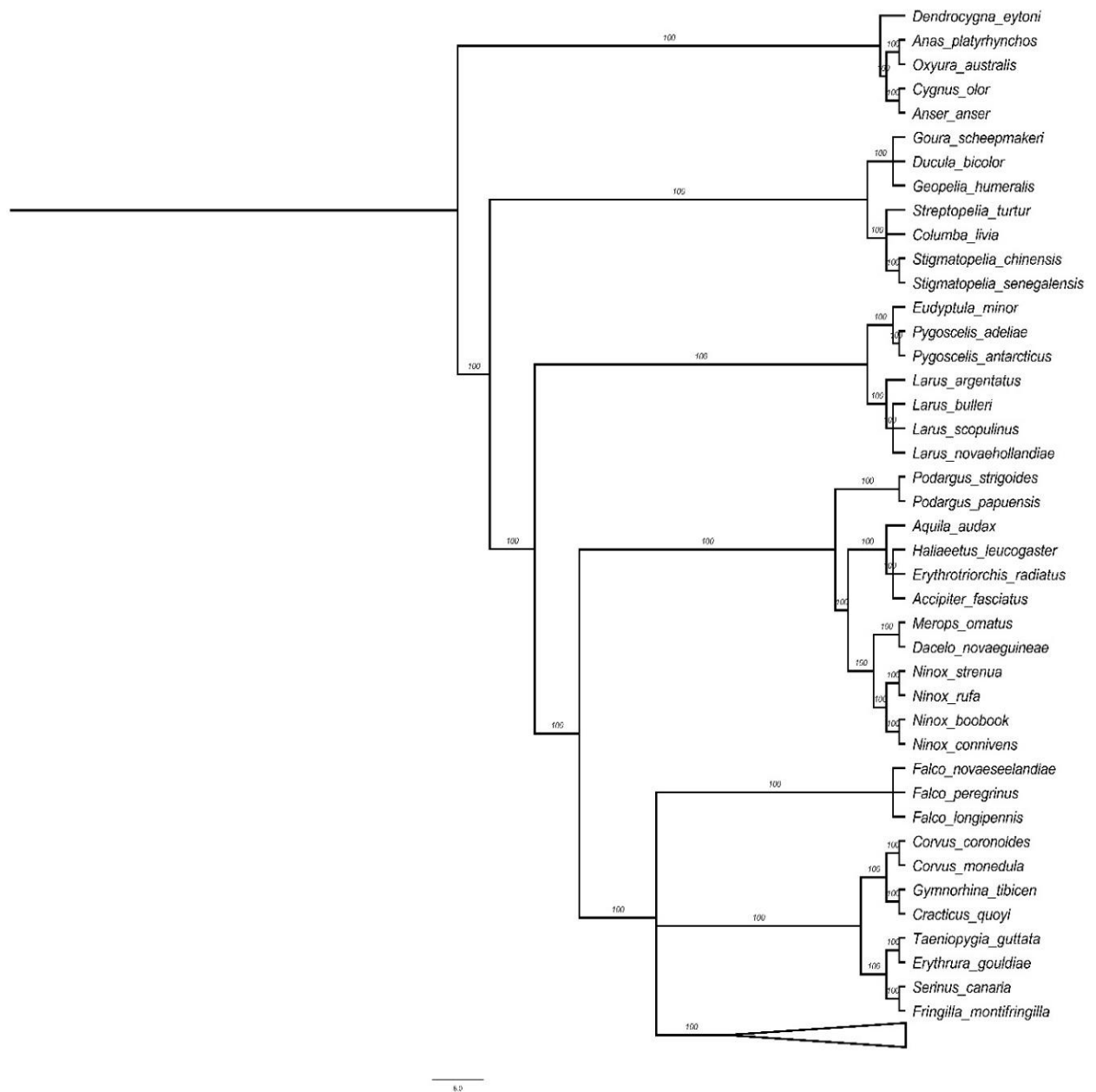
## Supplementary Materials

# Australasian Pigeon Circoviruses Demonstrate Natural Spillover Infection

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**Figure S1.** Avian host tree pruned from the BirdTree service (<https://birdtree.org>) to generate the host phylogeny. Consensus tree was obtained using consensus Tree Builder using Geneious Prime software (V.2022.1.1) with 98% Threshold support and 5% burn-in trees from a set of 5000 trees. The tree was rooted at midpoint and the branches were proportionally transformed. Consensus support for each branch was displayed as percent value (%) over the branch.



**Figure S2.** Avian host tree pruned from the BirdTree service (<https://birdtree.org>) to generate the host phylogeny. Consensus tree was obtained using consensus Tree Builder using Geneious Prime software (V.2022.1.1) with 98% Threshold support and 5% burn-in trees from a set of 5000 trees. The tree was rooted at midpoint and the branches were proportionally transformed. Consensus support for each branch was displayed as percent value (%) over the branch. The node belongs to Psittaciformes hosts has been collapsed in this tree.

**Table S1:** PCR detection of PiCV in Australia and Papua New Guinea

| Host                  | Species                          | Australia   |             | PNG         |            |
|-----------------------|----------------------------------|-------------|-------------|-------------|------------|
|                       |                                  | Bird Tested | Positive    | Bird Tested | Positive   |
| Racing Pigeon         | <i>Columba livia</i>             | 48          | 38          | 0           | 0          |
| Pied imperial pigeon  | <i>Ducula bicolor</i>            | 0           | 0           | 18          | 4          |
| Bar-shouldered dove   | <i>Geopelia humeralis</i>        | 0           | 0           | 10          | 2          |
| Scheepmaker's pigeon  | <i>Goura scheepmakeri</i>        | 0           | 0           | 8           | 2          |
| Senegal dove          | <i>Streptopelia senegalensis</i> | 2           | 1           | 0           | 0          |
| Spotted dove          | <i>Streptopelia chinensis</i>    | 2           | 1           | 0           | 0          |
| Rock pigeon           | <i>Columba livia</i>             | 10          | 4           | 0           | 0          |
| Plumed whistling duck | <i>Dendrocygna eytoni</i>        | 6           | 2           | 0           | 0          |
| Blue billed duck      | <i>Oxyura australis</i>          | 5           | 2           | 0           | 0          |
| Mallard duck          | <i>Anas platyrhynchos</i>        | 5           | 0           | 0           | 0          |
| Australian magpie     | <i>Gymnorhina tibicen</i>        | 4           | 2           | 0           | 0          |
| Total =               |                                  | 82          | 50 (60.98%) | 36          | 8 (22.22%) |

**Table S2:** PiCV partial *Rep* sequences obtained from the present study. Complete genome of PiCV found in this study is marked in bold.

| GenBank<br>accession no. | Year        | Isolate          | Location  | Species                                     | Source of samples  | Size of Sequences<br>(bp) |
|--------------------------|-------------|------------------|-----------|---|--------------------|---------------------------|
| OM470912                 | 2021        | CS21-0422        | NSW       | <i>Columba livia</i>                        | Liver and spleen   | 111                       |
| OM470913                 | 2021        | CS21-0422        | NSW       | <i>Columba livia</i>                        | Oropharynx         | 111                       |
| ON063538                 | 2013        | VDL-1            | PNG       | <i>Ducula bicolor</i>                       | Blood              | 113                       |
| ON063539                 | 2013        | VDL-2            | PNG       | <i>Ducula bicolor</i>                       | Blood              | 113                       |
| ON063540                 | 2013        | VDL-3            | PNG       | <i>Ducula bicolor</i>                       | Blood              | 113                       |
| ON063541                 | 2013        | VDL-4            | PNG       | <i>Geopelia humeralis</i>                   | Blood              | 113                       |
| ON063542                 | 2013        | VDL-5            | PNG       | <i>Goura scheepmakeri</i>                   | Blood              | 113                       |
| <b>MZ447864</b>          | <b>2019</b> | <b>CS19-1715</b> | <b>WA</b> | <b><i>Streptopelia<br/>senegalensis</i></b> | <b>Liver</b>       | <b>2039</b>               |
| ON086796                 | 2020        | CS20-0321        | NSW       | <i>Streptopelia<br/>chinensis</i>           | bursa of Fabricius | 110                       |
| ON086797                 | 2020        | CS20-1270        | NSW       | <i>Columba livia</i>                        | Blood              | 110                       |
| ON063537                 | 2020        | CS20-1270        | NSW       | <i>Columba livia</i>                        | Liver              | 110                       |
| MZ430510                 | 2020        | CS20-4427        | WA        | <i>Dendrocygna eytoni</i>                   | Blood              | 110                       |
| MZ430512                 | 2020        | CS20-4427        | WA        | <i>Dendrocygna eytoni</i>                   | Feather            | 699                       |
| MZ430509                 | 2020        | CS20-3773        | WA        | <i>Oxyura australis</i>                     | Blood              | 110                       |
| MZ430511                 | 2021        | CS21-0553        | QLD       | <i>Gymnorhina tibicen</i>                   | Feather            | 112                       |
| OR587845                 | 2021        | CS21-0422        | NSW       | <i>Columba livia</i>                        | Liver              | 432                       |
| OR587846                 | 2020        | CS20-4427        | WA        | <i>Dendrocygna eytoni</i>                   | Feather            | 502                       |
| OR587847                 | 2020        | CS20-1270        | NSW       | <i>Columba livia</i>                        | Blood              | 458                       |
| OR587848                 | 2020        | CS20-0321        | NSW       | <i>Streptopelia<br/>chinensis</i>           | bursa of Fabricius | 465                       |

**Table S3:** Ct value of DNA samples tested for PiCV qPCR.

| Sl. No. | VDL ID      | Sample type        | Host                  | PCR (P/N) | Ct value (qPCR) |
|---------|-------------|--------------------|-----------------------|-----------|-----------------|
| ID-1    | CS20-4427_1 | Blood              | Plumed whistling duck | P         | 38.74           |
| ID-2    | CS20-4427_2 | Feather            | Plumed whistling duck | P         | 30.74           |
| ID-3    | CS20-4427_3 | Feather            | Plumed whistling duck | N         | ND              |
| ID-4    | CS20-4427_4 | Blood              | Plumed whistling duck | N         | ND              |
| ID-5    | CS20-4427_5 | Feather            | Plumed whistling duck | N         | ND              |
| ID-6    | CS20-4427_6 | Feather            | Plumed whistling duck | N         | ND              |
| ID-7    | CS20-3773_1 | Blood              | Blue Billed Duck      | P         | 31.13           |
| ID-8    | CS20-3773_2 | Feather            | Blue Billed Duck      | P         | 31.1            |
| ID-9    | CS20-3773_3 | Feather            | Blue Billed Duck      | N         | ND              |
| ID-10   | CS20-3773_4 | Blood              | Blue Billed Duck      | N         | ND              |
| ID-11   | CS20-3773_5 | Feather            | Blue Billed Duck      | N         | ND              |
| ID-12   | CS21-0509_1 | Feather            | Mallard Duck          | N         | ND              |
| ID-13   | CS21-0509_2 | Feather            | Mallard Duck          | N         | ND              |
| ID-14   | CS21-0509_3 | Feather            | Mallard Duck          | N         | ND              |
| ID-15   | CS21-0509_4 | Feather            | Mallard Duck          | N         | ND              |
| ID-16   | CS21-0509_5 | Feather            | Mallard Duck          | N         | ND              |
| ID-17   | CS20-0321_1 | bursa of Fabricius | Spotted dove          | P         | 30.59           |
| ID-18   | CS20-0321_2 | Feather            | Spotted dove          | N         | ND              |
| ID-19   | CS21-0553_1 | Feather            | Australian magpie     | P         | 30.57           |
| ID-20   | CS21-0553_2 | Feather            | Australian magpie     | P         | 31.22           |
| ID-21   | CS21-0553_3 | Feather            | Australian magpie     | N         | ND              |
| ID-22   | CS21-0553_4 | Feather            | Australian magpie     | N         | ND              |
| ID-23   | CS20-1270_1 | Liver              | Rock Pigeon           | P         | 16.76           |
| ID-24   | CS20-1270_2 | Lung               | Rock Pigeon           | P         | 15.83           |
| ID-25   | CS20-1270_3 | Spleen             | Rock Pigeon           | P         | 29.46           |
| ID-26   | CS20-1270_4 | Blood              | Rock Pigeon           | P         | 18.66           |
| ID-27   | CS20-1270_5 | Blood              | Rock Pigeon           | N         | ND              |
| ID-28   | CS20-1270_6 | Blood              | Rock Pigeon           | N         | ND              |
| ID-29   | CS20_4890_1 | Cloacal swab       | Rock Pigeon           | N         | ND              |
| ID-30   | CS20_4890_2 | Cloacal swab       | Rock Pigeon           | N         | ND              |
| ID-31   | CS20_4890_3 | Cloacal swab       | Rock Pigeon           | N         | ND              |
| ID-32   | CS20_4890_4 | Cloacal swab       | Rock Pigeon           | N         | ND              |
| ID-33   | CS19-1715_1 | Liver              | Senegal dove          | P         | 18.78           |
| ID-34   | CS19-1715_2 | Feather            | Senegal dove          | N         | ND              |
| ID-35   | N/A         | Blood Spot         | Pied imperial pigeon  | P         | 32.98           |
| ID-36   | N/A         | Blood Spot         | Pied imperial pigeon  | N         | ND              |
| ID-37   | N/A         | Blood Spot         | Pied imperial pigeon  | N         | ND              |
| ID-38   | N/A         | Blood Spot         | Pied imperial pigeon  | N         | ND              |
| ID-39   | N/A         | Blood Spot         | Pied imperial pigeon  | N         | ND              |
| ID-40   | N/A         | Blood Spot         | Pied imperial pigeon  | N         | ND              |
| ID-41   | N/A         | Blood Spot         | Pied imperial pigeon  | P         | 32.01           |

|       |               |                    |                      |   |       |
|-------|---------------|--------------------|----------------------|---|-------|
| ID-42 | N/A           | Blood Spot         | Pied imperial pigeon | N | ND    |
| ID-43 | N/A           | Blood Spot         | Pied imperial pigeon | N | ND    |
| ID-44 | N/A           | Blood Spot         | Pied imperial pigeon | P | 29.39 |
| ID-45 | N/A           | Blood Spot         | Pied imperial pigeon | N | ND    |
| ID-46 | N/A           | Blood Spot         | Pied imperial pigeon | N | ND    |
| ID-47 | N/A           | Blood Spot         | Pied imperial pigeon | N | ND    |
| ID-48 | N/A           | Blood Spot         | Pied imperial pigeon | N | ND    |
| ID-49 | N/A           | Blood Spot         | Pied imperial pigeon | P | 31.42 |
| ID-50 | N/A           | Blood Spot         | Pied imperial pigeon | N | ND    |
| ID-51 | N/A           | Blood Spot         | Pied imperial pigeon | N | ND    |
| ID-52 | N/A           | Blood Spot         | Pied imperial pigeon | N | ND    |
| ID-53 | N/A           | Blood Spot         | Bar-shouldered dove  | P | 30.88 |
| ID-54 | N/A           | Blood Spot         | Bar-shouldered dove  | N | ND    |
| ID-55 | N/A           | Blood Spot         | Bar-shouldered dove  | N | ND    |
| ID-56 | N/A           | Blood Spot         | Bar-shouldered dove  | N | ND    |
| ID-57 | N/A           | Blood Spot         | Bar-shouldered dove  | N | ND    |
| ID-58 | N/A           | Blood Spot         | Bar-shouldered dove  | P | 31.44 |
| ID-59 | N/A           | Blood Spot         | Bar-shouldered dove  | N | ND    |
| ID-60 | N/A           | Blood Spot         | Bar-shouldered dove  | N | ND    |
| ID-61 | N/A           | Blood Spot         | Bar-shouldered dove  | N | ND    |
| ID-62 | N/A           | Blood Spot         | Bar-shouldered dove  | N | ND    |
| ID-63 | N/A           | Blood Spot         | Scheepmaker's pigeon | P | 31.68 |
| ID-64 | N/A           | Blood Spot         | Scheepmaker's pigeon | N | ND    |
| ID-65 | N/A           | Blood Spot         | Scheepmaker's pigeon | N | ND    |
| ID-66 | N/A           | Blood Spot         | Scheepmaker's pigeon | N | ND    |
| ID-67 | N/A           | Blood Spot         | Scheepmaker's pigeon | N | ND    |
| ID-68 | N/A           | Blood Spot         | Scheepmaker's pigeon | P | 30.86 |
| ID-69 | N/A           | Blood Spot         | Scheepmaker's pigeon | N | ND    |
| ID-70 | N/A           | Cloacal swab       | Scheepmaker's pigeon | N | ND    |
| ID-71 | CS 21-0422_1  | Liver              | Racing pigeon        | P | 15.22 |
| ID-72 | CS 21-0422_2  | Trachea            | Racing pigeon        | P | 17.85 |
| ID-73 | CS 21-0422_3  | bursa of Fabricius | Racing pigeon        | P | 16.38 |
| ID-74 | CS 21-0422_4  | Oropharynx         | Racing pigeon        | P | 12.58 |
| ID-75 | CS 21-0422_5  | Oro-cloacal swab   | Racing pigeon        | N | 33.62 |
| ID-76 | CS 21-0422_6  | Oro-cloacal swab   | Racing pigeon        | P | 27.46 |
| ID-77 | CS 21-0422_7  | Oro-cloacal swab   | Racing pigeon        | P | 31.02 |
| ID-78 | CS 21-0422_8  | Oro-cloacal swab   | Racing pigeon        | P | 28.6  |
| ID-79 | CS 21-0422_9  | Oro-cloacal swab   | Racing pigeon        | P | 29.92 |
| ID-80 | CS 21-0422_10 | Oro-cloacal swab   | Racing pigeon        | N | 33.01 |
| ID-81 | CS 21-0422_11 | Oro-cloacal swab   | Racing pigeon        | P | 27.25 |
| ID-82 | CS 21-0422_12 | Oro-cloacal swab   | Racing pigeon        | P | 31.55 |
| ID-83 | CS 21-0422_13 | Oro-cloacal swab   | Racing pigeon        | P | 31.07 |
| ID-84 | CS 21-0422_14 | Oro-cloacal swab   | Racing pigeon        | P | 29.07 |
| ID-85 | CS 21-0422_15 | Oro-cloacal swab   | Racing pigeon        | N | 32.19 |

|        |               |                    |               |   |       |
|--------|---------------|--------------------|---------------|---|-------|
| ID-86  | CS 21-0422_16 | Oro-cloacal swab   | Racing pigeon | P | 28.64 |
| ID-87  | CS 21-0422_17 | Oro-cloacal swab   | Racing pigeon | N | 31.64 |
| ID-88  | CS 21-0422_18 | Oro-cloacal swab   | Racing pigeon | P | 31.4  |
| ID-89  | CS 21-0422_19 | Oro-cloacal swab   | Racing pigeon | P | 30.83 |
| ID-90  | CS 21-0422_20 | Oro-cloacal swab   | Racing pigeon | P | 29.06 |
| ID-91  | CS 21-0422_21 | Oro-cloacal swab   | Racing pigeon | N | 32.29 |
| ID-92  | CS 21-0422_22 | Oro-cloacal swab   | Racing pigeon | P | 30.91 |
| ID-93  | CS 21-0422_23 | Oro-cloacal swab   | Racing pigeon | P | 31.21 |
| ID-94  | CS 21-0422_24 | Oro-cloacal swab   | Racing pigeon | P | 29.53 |
| ID-95  | CS 21-0422_25 | Oro-cloacal swab   | Racing pigeon | N | 31.99 |
| ID-96  | CS 21-0422_26 | Oro-cloacal swab   | Racing pigeon | P | 31.58 |
| ID-97  | CS 21-0422_27 | Oro-cloacal swab   | Racing pigeon | P | 18.2  |
| ID-98  | CS 21-0422_28 | Oro-cloacal swab   | Racing pigeon | P | 27.5  |
| ID-99  | CS 21-0422_29 | Oro-cloacal swab   | Racing pigeon | P | 14.46 |
| ID-100 | CS 21-0422_30 | Oro-cloacal swab   | Racing pigeon | N | 38.71 |
| ID-101 | CS 21-0422_31 | Spleen             | Racing pigeon | P | 13.27 |
| ID-102 | CS 21-0422_32 | Lung               | Racing pigeon | P | 21.97 |
| ID-103 | CS 21-0422_33 | Lymph node         | Racing pigeon | P | 15.65 |
| ID-104 | CS 21-0422_34 | Oro-cloacal swab   | Racing pigeon | P | 30    |
| ID-105 | CS 21-0422_35 | Oro-cloacal swab   | Racing pigeon | P | 22.73 |
| ID-106 | CS 21-0422_36 | bursa of Fabricius | Racing pigeon | P | 16.6  |
| ID-107 | CS 21-0422_37 | Oro-cloacal swab   | Racing pigeon | P | 28.88 |
| ID-108 | CS 21-0422_38 | bursa of Fabricius | Racing pigeon | P | 19.16 |
| ID-109 | CS 21-0422_39 | Oro-cloacal swab   | Racing pigeon | P | 27.54 |
| ID-110 | CS 21-0422_40 | Oro-cloacal swab   | Racing pigeon | P | 25.25 |
| ID-111 | CS 21-0422_41 | Oro-cloacal swab   | Racing pigeon | P | 24.79 |
| ID-112 | CS 21-0422_42 | Oro-cloacal swab   | Racing pigeon | P | 27.18 |
| ID-113 | CS 21-0422_43 | Oro-cloacal swab   | Racing pigeon | N | 38.01 |
| ID-114 | CS 21-0422_44 | Oro-cloacal swab   | Racing pigeon | P | 18.2  |
| ID-115 | CS 21-0422_45 | Oro-cloacal swab   | Racing pigeon | N | 33.19 |
| ID-116 | CS 21-0422_46 | Oro-cloacal swab   | Racing pigeon | N | 33.88 |
| ID-117 | CS 21-0422_47 | Oro-cloacal swab   | Racing pigeon | P | 31.29 |
| ID-118 | CS 21-0422_48 | Oro-cloacal swab   | Racing pigeon | P | 30.67 |

“P” denotes "Postive" and “N” denotes "Negative"; “ND” denotes “Not detected”; “N/A” denotes “Not applicable”