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Isolation of myxoviruses from dead birds arriving at Heathrow Airport, London

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SUMMARY

Forty-four haemagglutinating viruses were isolated from the pooled tracheal/cloacal swabs of the dead birds from 170 consignments of caged birds arriving at Heathrow Airport over a period of 6 months. Two isolates were identified as Newcastle disease virus but the remaining 42 were all identified as influenza viruses with Hav 7 Neq 2 antigens. All the consignments from which influenza viruses were isolated originated in India but had widespread destinations. The NDV isolates were from birds originating in central America and destined for Japan.

INTRODUCTION

Recent work on influenza viruses has emphasized the importance to both human and other animal populations of maintaining surveillance of the influenza virus types present in the avian species (Easterday & Couch, 1975). The transport of avian influenza viruses from one country to another may occur not only by free-flying migratory birds but also by the importation of caged birds (Slemons, Cooper & Orsborn, 1973*a*; Slemons, Johnson & Malone, 1973*b*; McFerran, Connor, Collins & Allan, 1974; Alexander, Allan, Harkness & Hall, 1974).

Examination of birds found dead in transit on world airlines at Heathrow Airport for influenza viruses offers an opportunity to assess the potential introduction of virus into Great Britain and many other countries that import exotic cage birds recently trapped from the wild.

MATERIALS AND METHODS

Viruses

All viruses were grown in 9- or 10-day-old embryonated eggs. Influenza reference strains were obtained from Dr G. Schild, World Influenza Centre, Mill Hill, London.

Table 1. *Details of consignments from which viruses were isolated*

Code	Flight arrival date	Isolation date	Type of dead birds in consignment	Airport of origin	Destination
A2	—	6. v. 76	Assorted finches	—	—
A6	—	6. v. 76	3 × Peking robin,* 12 × sibas, 3 × wagtails, 3 × buntings	—	—
A7	—	6. v. 76	8 × buntings, 15 × tricoloured nuns, 6 × tiger finches	—	—
A9	—	6. v. 76	Assorted finches	—	—
A11	—	6. v. 76	Assorted finches	—	—
A15	—	13. v. 76	12 × spice birds, 3 × bulbirds	—	—
A22	—	21. v. 76	8 × spice birds, 1 × tiger finch, 4 × tricoloured nuns	'India'	Amsterdam
A23	—	21. v. 76	17 × tricoloured nuns	—	—
A27	—	21. v. 76	26 × assorted finches	—	—
A29	—	21. v. 76	Assorted finches	—	—
A32	—	21. v. 76	48 × tricoloured nuns, 5 × spice birds, 1 × tiger finch	—	—
A34	—	9. vi. 76	7 × tricoloured nuns, 3 × blossom-headed parakeets	Calcutta	Frankfurt
A42	—	9. vi. 76	4 × plum-headed parakeets,† 4 × finches	Calcutta	Frankfurt
A44	—	29. vi. 76	2 × ring-necked parakeets, 6 × Alexandrine‡	Calcutta	Palma
A45	—	29. vi. 76	11 × tricoloured nuns	Calcutta	Amsterdam
A49	—	29. vi. 76	2 × tricoloured nuns, 1 × Alexandrine, 3 × mynah birds, 2 × plum-headed parakeets	Delhi	Naples
A52	—	29. vi. 76	5 × assorted parakeets, 5 × assorted finches	Delhi	Naples
A53	—	29. vi. 76	12 × shamas,§ 1 × Peking robin, 6 × assorted finches	'India'	Copenhagen
A54	—	2. vii. 76	6 × blossom-headed parakeets, 8 × tricoloured nuns, 38 × white eyes‖	Calcutta	'Germany'
A58	—	2. vii. 76	3 × mynah birds	—	—
A64	—	7. vii. 76	2 × mynah birds, 4 × tiger finches, 1 × tricoloured nuns	—	—
A65	—	7. vii. 76	3 × tricoloured nuns, 7 × mynah birds	—	—
A67	—	7. vii. 76	65 × tricoloured nuns, 4 × spice birds, 1 × green finch	—	—
A70	—	7. vii. 76	49 × tricoloured nuns	—	—
A71	—	7. vii. 76	37 × assorted finches	Calcutta	—
A74	—	7. vii. 76	37 × tricoloured nuns	Calcutta	—
A80	9. vii. 76	20. vii. 76	5 × blossom-headed parakeets	Calcutta	Amsterdam
A81	6. vii. 76	20. vii .76	10 × assorted finches	Calcutta	Amsterdam
A85	8. vii. 76	20. vii. 76	14 × ring-necked parakeets	Calcutta	Amsterdam
A87	9. vii. 76	20. vii. 76	3 × ring-necked parakeets	Calcutta	Amsterdam
A89	30. vii. 76	3. viii. 76	4 × mynah birds	Calcutta	—
A95	31. vii. 76	7. viii. 76	Mynah birds	Calcutta	—
A102	14. viii. 76	7. ix. 76	3 × mynah birds	Calcutta	Rome
A104	—	7. ix. 76	5 × spice birds, 3 × silver bills,¶ 11 × tiger finches, 10 × tricoloured nuns	Calcutta	Rome
A109	—	7. ix. 76	Spice birds, silver bills, tiger finches, tricoloured nuns	Calcutta	Milan
A112	6. ix. 76	19. x. 76	29 × tricoloured nuns, 14 × spice birds	Calcutta	Lyon

Table 1 (*cont.*)

Code	Flight arrival date	Isolation date	Type of dead birds in consignment	Airport of origin	Destination
A114	5. ix. 76	19. x. 76	21 × tricoloured nuns, 5 × spice birds	Calcutta	Lyon
A120	13. ix. 76	19. x. 76	47 × white eyes, 4 × spice birds, 1 × yellow-winged bulbill, 1 × leaf bird	Calcutta	'Germany'
A145	22. ix. 76	26. x. 76	6 × blossom-headed parakeets	Calcutta	—
A146	22. x. 76	18. xi. 76	127 × assorted finches	Calcutta	Brussels
A151	22. x. 76	18. xi. 76	27 × assorted finches	Calcutta	Brussels
A155	6. x. 76	24. xi. 76	4 × ring-necked parakeets	Calcutta	Rome
A160 NDV	30. xi. 76	10. xii. 76	1 × yellow and blue macaw,** 1 × green-winged macaw,†† 1 × yellow-crowned amazon‡‡	Panama	Tokyo
A161 NDV	30. xi. 76	10. xii. 76	1 × yellow and blue macaw, 1 × green-winged macaw, 1 × scarlet macaw§§	Panama	Tokyo

* *Leiothrix lutea lutea.*
† *Psittacula cyanocephala.*
‡ *Psittacula eupatria nipalensis.*
§ *Copsychus malabaricus indicus.*
‖ *Zosterops palpebrosa palpebrosa.*
¶ *Enodice malabarica malabarica.*
** *Ara ararauna.*
†† *Ara chloropetra.*
‡‡ *Amazona ochrocephala.*
§§ *Ara macao.*

Antisera and titrations

Antisera were prepared in chickens and titrations done as described (Alexander *et al.* 1974).

Sampling procedure

Only dead birds were examined. The procedure was to remove all dead birds from a single consignment (which frequently contained more than one species) into a plastic bag which was then sealed and held at −20 °C. Consignments of dead birds were collected at approximately fortnightly intervals from Heathrow Airport and transported to the Central Veterinary Laboratory for virus isolation.

Virus isolation

Virus isolation procedures were restricted to swabbing the trachea and cloaca of each bird and pooling all the swabs for each sealed consignment into antibiotic broth. When a consignment consisted of a large number of birds only a representative proportion, usually 50% but at least 20%, were swabbed. The broth into which the swabs were placed contained Gentamicin (Flow Laboratories Ltd) 100 μg/ml and Terramycin (Pfizer Ltd) 500 μg/ml.

Five 9- or 10-day-old specific-pathogen-free embryonated eggs were each inoculated with 0·2 ml of the antibiotic broth from each consignment. The allantoic/amniotic fluids from dead eggs and eggs still alive 4 days after inoculation were tested for haemagglutinin activity. Only one egg passage was used for virus isolation.

Identification of birds

Birds were identified by reference to standard textbooks. Generally no attempt was made to distinguish the various species of finches or large batches of similar birds such as parakeets.

RESULTS

Birds received

During the 6-month period under report, 170 consignments containing more than 4500 dead birds were examined. Excluding the different species of finches, these consignments have contained 54 different species. Finches were the most common bird examined, 43 consignments contained a total of 1540 finches. The next most frequently seen birds were tricoloured nuns (*Munia malacca malacca*), 31 consignments, 690 birds, followed by: ring-necked parakeets (*Psittacula krameri krameri*) 23 and 102, mynah birds (*Aeridotteres tristis*) 22 and 117, spice birds (*Munia punctulata punctulata*) 21 and 256, and blossom-headed parakeets (*Psitticula roseata roseata*) 17 and 74. The number of dead birds from a single consignment varied from one budgerigar or one crane to 921 finches. Usually the number of dead birds did not exceed 10% of the consignment but occasionally much higher proportions were found. No attempt was made to discover the cause of death of the birds.

Isolation of virus

Haemagglutinating virus was isolated from the pooled swabs of 44 of the 170 consignments examined. In two consignments the virus was Newcastle disease virus but in the remaining 42 the virus proved to be influenza virus of Hav 7 Neq 2 subtype. The details of the consignments from which viruses were isolated are shown in Table 1. The species most frequently forming part of virus-positive consignments were: tricoloured nuns, 17; finches, 19; spice birds, 9; mynah birds, 7.

Where known the airports of both origin and destination are included in Table 1. The influenza virus-positive consignments were exclusively from the Indian sub-continent but were in transit to several widespread European destinations.

DISCUSSION

Influenza A viruses of the Hav 7 Neq 2 subtype were isolated from the dead birds of approximately 25% of the 170 consignments tested. It is possible that this frequency may be somewhat artificially high owing to contamination of different consignments examined on the same day despite the precautions taken to avoid this. Even allowing for this possibility the results represent a high incidence of influenza A in birds exported from the Indian sub-continent.

Reported isolates of influenza viruses from cage birds have been of Hav 1, Hav 3, Hav 4 and Hav 5 subtypes (Butterfield, Yedlouschnig & Dardiri, 1973; Slemons *et al.* 1973*a*, *b*; McFerran *et al.* 1974; Alexander *et al.* 1974), although influenza A viruses of Hav 7 Neq 2 subtype have been isolated from feral birds in the USSR (Webster, Isachenko & Carter, 1974).

There is no evidence to suggest that the influenza viruses isolated from the dead

birds were in any way responsible for the death of the birds and no virus was isolated from 75% of the consignments. However, in conditions of stress which may be expected in the caging on shipping of wild birds, infection with even non-pathogenic influenza viruses may have contributed to the death of the birds.

Epizootiologically the isolations reported in this study are of less importance in relation to the prevalence of virus in the country of origin than they are in terms of the introduction of virus into Great Britain and other countries. It is likely that influenza virus of the type Hav 7 Neq 2 is endemic amongst birds in the Indian sub-continent, but the mixing of birds of different species and from different geographical locations in transit, may render this conclusion erroneous.

The possible introduction of avian influenza viruses into a country may be important not only to the avian species of that country but also to other animals including man (Webster *et al.* 1974). This is particularly so in the case of isolations of the Hav 7 subtype in view of the reported relationships between the Hav 7 subtype, the human influenza A subtype H3 and the equine subtype Heq 2 (Laver & Webster, 1973; Webster *et al.* 1974).

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