

# Host response differences among 5 avian species to an influenza virus—A/turkey/Ontario/7732/66 (Hav5N?)\*

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*Experiments were carried out to determine how turkeys, ducks, quails, pheasants, and pigeons respond to the same avian influenza virus (A/turkey/Ontario/7732/66). The factors considered were the antibody response, virus shedding, and overt signs of disease. While no signs of disease were observed among the pheasants and ducks, turkeys had severe disease that terminated fatally. There was a marked antibody response in the pheasants and quail and a poor response in ducks. The ease and regularity of virus recovery varied considerably. The marked differences between species in the response to infection with avian influenza viruses should be taken into account in the design and interpretation of studies of the role of avian species in the natural history of influenza.*

Demonstrable antigenic relationships and genetic recombination between avian and human type A influenza viruses have led to considerable speculation about the possibility of interspecies transfer of influenza virus (Pereira, 1969). Of particular interest has been the role of wild birds in the dissemination of the virus. Antibody against influenza virus A has been demonstrated in wild avian species (Easterday et al., 1968; Dasen & Laver, 1970; Winkler et al., 1972) and one virus strain has been isolated from terns (Becker, 1963).

This study was undertaken to determine the response of various avian species following exposure to an influenza virus isolated from, and highly pathogenic for, turkeys. Of particular interest were the signs of disease, the shedding of virus, and the nature of the antibody response.

## MATERIALS AND METHODS

### Viruses

Influenza virus A/turkey/Ontario/7732/66 (Hav5N?) was obtained from Dr Gerhard Lang, University of Guelph, Ontario, Canada. A lyophilized sample was re-suspended in sterile, twice-distilled water and diluted 1:1000 in saline and 0.2-ml amounts were inoculated into the allantoic sac of 10-day-old embryonated chicken eggs. After 40 hours of incubation at 37°C, the eggs were chilled and allantoic fluids were

collected, pooled, and frozen at -50°C to be used as reference stock. Working stock was produced by making one more egg passage in a similar manner.

Infectivity endpoints expressed as 50% embryo infective dose (EID<sub>50</sub>) were calculated by the method of Reed & Muench. Assay for EID<sub>50</sub> was accomplished by using 4 eggs per 10-fold dilution of virus, 0.1 ml of diluted virus being inoculated into the allantoic cavity of each egg. The diluent used was brain-heart infusion broth (BHIB). Inoculated eggs were incubated for 32 hours at 37°C; the eggs were then chilled and 0.5-ml amounts of allantoic fluid from each egg were placed in disposable plastic trays and 0.5 ml of 0.5% chicken red blood cells was added to each well.

### Experimental animals

The avian species exposed were 6-month-old female Beltsville Broadwhite turkeys, 5-month-old male and female Japanese quails (*Coturnix coturnix japonica*), 8-month-old female ringneck pheasants, 8-month-old male and female mallard ducks, and 2-3-year-old male and female pigeons. The turkeys and pheasants were housed in individual isolators. The quails, pigeons, and ducks were housed separately in groups. All the exposed birds were kept in isolation.

### Exposure and sample collection

*Exposure.* All birds were exposed to virus by the administration of intranasal drops: this pro-

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cedure resulted in virus being swallowed and inhaled. The dose range given to each species is shown in Table 1.

**Tracheal swabs.** Tracheal swabs collected from each bird prior to exposure and at different intervals after exposure were placed in 1.0 ml of BHIB containing 800 IU of penicillin and 623 IU of streptomycin. The inoculated broth was allowed to stand at room temperature for approximately 1 hour and was then stored at  $-60^{\circ}\text{C}$ ; it was thawed immediately prior to inoculation into 10-day-old embryonated chicken eggs for attempted virus recovery. Cotton-wool swabs were used on turkeys, ducks, and pheasants. Soft pliable copper wire folded double and twisted with a small loop 2 mm in diameter was used for swabbing pigeons and quails.

**Serum samples.** Serum samples were collected from each bird prior to exposure and at approximately weekly intervals following exposure. The samples were stored at  $-20^{\circ}\text{C}$  until they were examined for antibody by the haemagglutination inhibition (HI) and agar gel diffusion (AGD) tests.

### Serology

**Haemagglutination inhibition tests:** The level of HI antibody in serum specimens was measured by standard methods in plastic trays (WHO Expert Committee on Respiratory Virus Diseases, 1959).

Endpoints were read when 100% of the haemagglutinating activity of the antigen was inhibited.

**Agar gel diffusion.** Antibody against the type-specific antigen (ribonucleoprotein [RPN<sup>3</sup>] or "S" antigen) of influenza virus was detected by the agar gel diffusion (AGD) techniques described by Beard (1970), with the following modifications: (1) the antigen was prepared by inoculating a  $10^{-3}$  dilution of PR8 into the allantoic cavity of 10-day-old embryonated chicken eggs and incubating for 18 hours at  $37^{\circ}\text{C}$ , and (2) 0.45% Ionagar No. 2 was used.

### RESULTS

After infection with turkey/Ontario/7732/66 virus the mortality rate among birds of different species varied from zero to 100%. All birds that developed signs of disease eventually died. All infected turkeys died and showed signs of severe diarrhoea, depression, impaired righting reflex, and torticollis before death. In other experiments the  $\text{ID}_{50}$  and  $\text{LD}_{50}$  for turkeys were very similar if not identical. Of 20 quail, 3 (15%) died following signs of torticollis and depression. Of 19 pigeons, 1 (approx. 5%) died, the only sign of disease being depression 1 day before death. No signs of disease were seen in exposed ducks and pheasants (1 pheasant had increased respiratory sounds that could have been

Table 1. Summary of results of exposure of avian species to turkey/Ontario/7732/66 virus, by intranasal inoculation

Species	No. of birds exposed	Age (months)	$\text{EID}_{50}$ and route <sup>a</sup>	Housing	Signs of disease	Virus isolations <sup>b</sup>	HI test <sup>c</sup>	AGD test <sup>d</sup>
turkey	4	6	$3.1 \times 10^4$ – $2.2 \times 10^5$	isolated individuals	severe depression, torticollis, diarrhoea	4/4	all birds died	
quail	20	5	$3.1 \times 10^3$ – $2.2 \times 10^4$	one group	torticollis in birds that died	15/20 (three birds died)	17/20	12/20
pheasant	14	8	$1.7 \times 10^4$ – $1.1 \times 10^5$	isolated individuals	none	12/14	13/14	13/14
duck	18	8	$2.6 \times 10^4$ – $1.6 \times 10^5$	four groups	none	8/18	16/18	0/18
pigeon	19	24-36	$8.5 \times 10^3$ – $1.1 \times 10^5$	two groups	depression in the one bird that died	2/19	17/19 (one bird died)	17/19 (one bird died)

<sup>a</sup>  $\text{EID}_{50}$  = infectivity endpoint expressed as 50% chicken embryo infective dose, calculated by method of Reed & Muench.

<sup>b</sup> 4/4 indicates that all 4 exposed birds were positive for virus recovery in one or more tracheal swabs out of the 4 birds exposed.

<sup>c</sup> 17/20 indicates that 17 of the 20 birds exposed developed HI antibody.

<sup>d</sup> 12/20 indicates that 12 of the 20 birds exposed developed anti-RNP antibody.

the result of trauma after swabbing). The results of these experiments are summarized in Table 1.<sup>1</sup>

There was marked variation between the species in the number of exposed birds from which virus could be recovered on one or more days. The proportions of the total number of birds from which virus was recovered were as follows: turkeys, 100%; pheasants, 80%; quails, 75%; ducks, 45%; and pigeons approximately 10%.

The antibody responses of infected birds, as indicated by the results of HI and AGD tests, are summarized in Table 1. The responses were strongest in pheasants and quails. One pigeon produced only questionable positive results in the HI and AGD tests; 16 of 18 ducks had low levels of HI antibody but anti-RNP antibody could not be detected in the AGD test.

#### DISCUSSION

Differences in the biological behaviour of particular avian influenzaviruses in different hosts have been subjected to only limited investigation. However, distinct biological differences have been recognized among the highly pathogenic strains of avian influenzavirus—e.g., fowl plague, chicken/Scotland/59, tern/South Africa/61, and turkey/Ontario/7732/66. These viruses have been shown to be highly pathogenic for one or more avian species. Fowl plague was found to be highly pathogenic for several species, and subsequently strains shown to be pathogenic for one particular

<sup>1</sup> Tables giving the results in separate species have been deposited in the WHO Library and photocopies may be obtained on request to Chief Librarian, World Health Organization, 1211 Geneva 27, Switzerland.

avian species have been assumed to be so for others. This assumption may be misleading as the host range of these viruses may be quite limited and not as extensive as that of classical fowl plague. In using serological methods in attempting to determine whether wild birds have had previous experience with influenzaviruses, the tendency has been to expect similar immune responses in all avian species. Even though avian species are known to have a well developed antibody response the response to avian influenza of a specific species, especially among wild birds, has not been explored extensively.

The results presented here indicate that there is considerable variation among species in response to a single strain of avian influenzavirus.

While turkey/Ontario/7732/66 is highly pathogenic for turkeys, it is markedly less so for quails, pheasants, ducks, and pigeons. Signs of disease could not be observed in pheasants and ducks. Because of the lack of evidence of an immune response it may even be questioned whether the ducks were infected.

Narayan et al. (1969) found, in transmission experiments, that turkey/Ontario/7732/66 caused an acute disease in chickens and turkeys, but was non-pathogenic to ducks, geese, and pigeons.

In experiments not reported here, we have observed good HI antibody responses (160–2 560) when quails were exposed to quail/Italy/544/66 (Hav2N?), but much lower HI antibody responses (10–40) when they were exposed to turkey/Wisconsin/68 (Hav5N?). Leslie & Benedict (1969) noted that following injection of bovine serum albumin (BSA) quails responded very poorly when antibodies were measured by radio-immunoelectrophoresis techniques.

#### ADDENDUM

An additional set of experiments was performed to determine how the same five species—turkeys, pheasants, ducks, quails, and pigeons—would respond to another avian influenzavirus (A/turkey/Wisconsin/68, Hav5N?) that is closely related antigenically to the A/turkey/Ontario/7732/66 virus. Both viruses have the Hav5 haemagglutinin.

There were no deaths and no signs of disease in any of the five species when they were exposed to the A/turkey/Wisconsin/68 virus in a manner similar to that described for the A/turkey/Ontario/7732/66 virus. Some individuals of all species were infected as

determined by the recovery of virus and/or the agar gel diffusion test (AGDT). There was considerable variation among the species in the number of exposed birds from which virus could be recovered. The highest levels of antibody as determined by the haemagglutination-inhibition test (HIT) were in the pheasants and turkeys. Similarly, the number of positive AGDT was greater among pheasants and turkeys. A large proportion of the quail and duck sera were HIT-positive at low levels but very few of the sera were positive in the AGDT. The results are given in Table 2, and the differences in the responses of one

Table 2. Results of exposure of avian species to turkey/Wisconsin/68 virus <sup>a</sup>

Species	No. of birds exposed	Age (months)	Infecting dose (EID <sub>50</sub> )	Housing	Virus recovery	HIT	AGDT	NIT <sup>b</sup>
turkey	7	10	3 × 10 <sup>4</sup>	1 group	4/7	5/7	4/7	+
pheasant	14	10	7.5 × 10 <sup>4</sup>	2 exposed per cage	13/14	14/14	13/14	+
quail	16	8	1 × 10 <sup>4</sup>	1 group	8/16	13/16	2/16	-
duck	19	11	3.17 × 10 <sup>5</sup>	4 groups	15/19	16/19	2/19	+
pigeon	16	unknown	1.6 × 10 <sup>5</sup>	4 groups	3/16	9/16	0/16	-

<sup>a</sup> None of the birds showed signs of disease.

<sup>b</sup> Results of the neuraminidase-inhibition tests: +, one or more individual birds with titre ≥ 10; - one or more individual birds with titre of < 10.

Table 3. Differences in responses of birds following exposure to two viruses

Species	Criterion	Virus	
		ty/Ont/7732/66	ty/Wis/68
turkey	mortality rate (%)	100	0
quail	HIT titre	≥ 80	≤ 40
quail	no. AGDT-positive/total exposed	12/17 (3 died)	2/16
duck	virus recovery/total exposed	8/18	16/19
duck	no. AGDT-positive/total exposed	0/18	2/19
duck	positive sera in NIT	no	yes
pigeon	no. HIT-positive/total exposed	1 ± /19	6/19
pheasant	all criteria	no apparent differences	

species to two antigenically related viruses are summarized in Table 3.

Sera from the different species were tested for the presence of neuraminidase-inhibiting (NI) antibody against both viruses as described by Webster & Pereira (1968). Pheasants and quails infected with the turkey/Ontario/7732/66 virus had NI antibody titres of greater than 10, and pheasants, turkeys, and ducks infected with turkey/Wisconsin/68 virus also had NI

antibody levels of greater than 10. In both cases the highest levels of NI antibody were in the pheasants.

In addition to providing further demonstration of the fact that several avian species do not respond similarly to the same virus, these experiments also show that one species cannot be expected to respond to two antigenically related influenzaviruses in the same manner.

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## RÉSUMÉ

## DIFFÉRENCES DE RÉACTION, SELON L'HÔTE, À L'INFECTION PAR UN VIRUS GRIPPAL, A/TURKEY/ONTARIO/7732/66 (HAV5N?), CHEZ 5 ESPÈCES AVIAIRES

On a étudié chez 5 espèces d'oiseaux (4 dindes, 20 cailles, 14 faisans, 18 canards et 19 pigeons) la réponse à l'infection expérimentale par un même virus de la grippe aviaire (A/turkey/Ontario/7732/66).

L'infection a entraîné la mort de toutes les dindes. Trois cailles (15%) et 1 pigeon (5%) ont succombé. Les faisans et les canards n'ont présenté aucun signe de maladie. Le virus a été retrouvé chez toutes les dindes, chez la plupart des cailles (15/20) et des faisans (12/14) et chez environ 50% des canards et 10% des pigeons.

La réponse immunitaire a été très variable suivant les espèces. Les faisans ont fourni les titres les plus élevés d'anticorps inhibant l'hémagglutination, alors que chez les canards la production d'anticorps est restée relativement faible. L'épreuve de diffusion en gel de gélose a permis de déceler des anticorps anti-ribonucléoprotéine dans la quasi-totalité des sérums de faisans et dans un certain nombre de sérums de cailles.

Ces résultats montrent la grande variabilité de la réceptivité de diverses espèces d'oiseaux à l'infection par un même virus de la grippe aviaire.

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