

# A new subtype of type A influenzavirus isolated from turkeys

G. LANG,<sup>1</sup> BELA TUMOVÁ,<sup>2</sup> & G. C. SCHILD<sup>3</sup>

*A new subtype of avian influenzavirus A was isolated in January 1967 from an epizootic in a turkey hatchery in Ontario, Canada. The disease was fatal in 65 of 2 500 hens involved. Virus was isolated from lung and trachea tissue of three dead turkeys. Sera from convalescent birds contained antibody against the viruses isolated from the outbreak but not against other known type A avian influenzaviruses, Newcastle disease virus, Myxovirus Yucaipa, or Mycoplasma gallisepticum.*

*The strain designated A/turkey/Ontario/6118/67 contained the influenza A type ribonucleoprotein. Haemagglutinin and neuraminidase antigens of the strain differed antigenically from the envelope antigens of other avian influenzaviruses isolated from birds, horses, pigs, or man. The designation of turkey/Ontario/6118/67 as containing haemagglutinin of avian subtype 8 (Hav 8) and neuraminidase of avian subtype 4 (Nav 4) is proposed.*

Virological investigations carried out during the last two decades have revealed that birds are naturally susceptible to infection by several different serotypes of influenzavirus A (Pereira, 1969; Easterday & Tumová, 1972). This paper reports the isolation and identification of a new immunological variant of this virus type.

## MATERIALS AND METHODS

### Viruses

The strains employed in this study are listed in Tables 1 and 2: their history and characteristics have been described previously (Pereira, 1969; Easterday & Tumová, 1972). All the strains were propagated in embryonated eggs and the allantoic fluid was used as a source of the virus for serological studies.

### Sera

Sera from convalescent ferrets, turkeys, ducks, and chickens and from hyperimmune rats were used for the haemagglutination inhibition (HI) tests. Sera

were treated with receptor destroying enzyme (RDE) according to the method described by the WHO Expert Committee on Respiratory Viruses (1959). Hyperimmune rabbit and rat sera (Schild & Newman, 1969; Tumová & Easterday, 1969) were used in the neuraminidase inhibition (NI) tests.

### Serology

The haemagglutination inhibition (HI) tests and complement fixation (CF) tests were performed in plastic trays using conventional techniques (WHO Expert Committee on Respiratory Viruses, 1959; Pereira et al., 1964). The neuraminidase inhibition (NI) tests were performed according to the method described by Webster & Pereira (1968). The immunodiffusion tests were performed as described by Beard (1970).

## RESULTS

### History of the outbreak

In January 1967, a turkey breeder in Ontario, Canada, noticed a marked fall in egg production of three laying flocks. About 2 500 35-week-old hens were affected. Concomitantly with the fall in egg production, the birds were depressed for 3-4 days and 65 of them died during the acute stage of the disease. Serofibrinous inflammation of the serous membranes of chest and abdomen were the out-

<sup>1</sup> Ontario Veterinary College, Guelph, Ontario, Canada. Present address: P.O. Box 91, Lumumbashi, Zaire.

<sup>2</sup> Institute of Epidemiology and Microbiology, Prague, Czechoslovakia.

<sup>3</sup> World Influenza Centre, National Institute for Medical Research, London, England.

Table 1. Results of haemagglutination inhibition tests with turkey/Ontario 6118/67 and avian influenzaviruses

Haemagglutinin subtype	Antisera	HI titres <sup>a</sup>		Laboratories <sup>b</sup>
		Homologous virus	Ontario 6118	
Hav 1	FPV (Dutch strain)	320	<10	L
	turkey/England/63	640	<10	P
Hav 2	chicken/Germany/N/49	640-1 280	<10	P, L
	duck/Manitoba/53	320-640	<10	G, P
	quail/Italy/1117/65	320-1 280	<10	G, P, L
	quail/Italy/544/66	1 280	<10	P
	duck/Italy/574/66	640	<10	P
	duck/Italy/946/66	320	<10	P
	pheasant/Italy/647/66	640-1 280	<10	P, L
Hav 3	duck/England/56	120-1 280	<10	P, L
Hav 4	duck/Czechoslovakia/56	80-240	<10	G, P, L
	duck/England/62	120-320	<10	G, P, L
	turkey/Alberta/6962/66	80-160	<10	G, P
	turkey/Ontario/3963/68	320	<10	G
Hav 5	chicken/Scotland/1/59	320-1 280	<10	G, P, L
	tern/S. Africa/61	160-320	<10	P, L
	turkey/Ontario/6213/66	320-640	<10	G, P, L
	turkey/Ontario/7732/66	1 280	<10	G
	turkey/Ontario/6828/67	640-1 280	<10	G, P, L
	turkey/Ontario/5265/66	320	<10	G
	turkey/Wisconsin/68	320-1 280	<10	P, L
	duck/Pennsylvania/69	640	<10	L
Hav 6	turkey/Ontario/3724/63	30-160	<10	G, P, L
	turkey/California/64	160	<10	P, L
	turkey/Ontario/5510/64	80	<10	G
	turkey/Ontario/5050/65	40	<10	G
	turkey/Massachusetts/65	160-320	<10	G, P, L
	turkey/Wisconsin/66	240-640	<10	G, P, L
	turkey/Ontario/5379/66	160	<10	G
	turkey/England/66	240-320	<10	P, L
	turkey/Ontario/5021/67	80	<10	G
	turkey/Ontario/4054/68	40	<10	G
	turkey/Ontario/3579/69	160	<10	G
	turkey/Ontario/2614/70	320	<10	G
	Hav 7	duck/Ukraine/1/63	30-160	<10
Hav 8	turkey/Ontario/6118/67 <sup>c</sup>		320-640	G, P, L

<sup>a</sup> Titres given as reciprocals of the serum dilution.

<sup>b</sup> Laboratories in Guelph, Ontario (G); Prague, Czechoslovakia (P); and London, England (L).

<sup>c</sup> Serum turkey/Ontario 6118/67 failed to inhibit the haemagglutination of the avian influenza viruses subtype 1-7.

standing lesions noted at necropsy. The disease episode, involving rapidly one flock after another, lasted for about 3 weeks. The daily egg yield, which had dropped from 60% to 14%, returned slowly to 56%; recovery was slow and about 100 birds had to be culled.

#### Isolation of the virus

Three virus isolations were attempted yielding the three isolates 6118, 6120, and 6186. The isolations were made by allantoic inoculation into embryonated hen's eggs of lung and trachea specimens from dead turkeys submitted 2, 5, and 7 days

Table 2. Results of HI tests with turkey/Ontario/6118/67 virus and other strains of the myxovirus group

Myxovirus (H subtype)	Antisera	HI titres <sup>a</sup>		Laboratories <sup>b</sup>
		Homologous virus	Ontario/6118	
Influenzavirus				
equine (Heq1)	equine/Prague/56	80-310	<10	G, P, L
(Heq2)	equine/Miami/63	160	<10	G, P, L
porcine (Hsw1)	swine/Iowa/15/30	160-640	<10	G, P, L
	swine/Cambridge/39	1 280	<10	L
	swine/Wisconsin/61	640	<10	L
	swine/Manitoba/63	320	<10	G
human (H0)	A/Puerto Rico/8/34	160-320	<10	G, P, L
	A/England/WS/33	160	<10	L
(H1)	A/New Jersey/FM1/46	640	<10	P, L
	A/England/1/55	640	<10	L
(H2)	A/Singapore/1/57	1 280	<10	P, L
	A/England/12/64	2 560	<10	L
	A/Tokyo/3/67	320	<10	L
(H3)	A/Hong Kong/1/68	5 120	<10	P, L
Newcastle disease virus	chicken/Ontario/1950	640	<10	G
	vaccine strain B1	160	<10	G
Myxovirus Yucaipa	chicken/California/60	160	<10	G, P
	turkey/Ontario/5724/67	320	<10	G
	turkey/Alberta/2654/67	160	<10	G
Parainfluenza	turkey/Ontario/4538/67 <sup>c</sup>	80	<10	G, P
	turkey/Wisconsin/68	160	<10	P
	turkey/Ontario/6661/67	160	<10	P

<sup>a</sup> Titres given as reciprocals of serum dilution.

<sup>b</sup> Laboratories in Guelph, Ontario (G); Prague, Czechoslovakia (P); and London, England (L).

<sup>c</sup> New myxoviruses isolated from turkeys.

after the beginning of the outbreak. The presence of virus in the eggs resulted in embryo mortality (2-6 days after inoculation) and the presence of chicken cell haemagglutinins in the allantoic fluid. Specific lesions were not noted in infected embryos or their annexes.

#### Serology with convalescent turkey sera

Sera taken from convalescent birds at different intervals following the epizootic were free from HI antibodies to Newcastle disease virus, Myxovirus Yucaipa, avian influenza (turkey/Alberta 6962/66, turkey/Ontario 6213/66 (Hav5N?), turkey/Ontario 7732/66 (Hav5N?), and turkey/Ontario 3724/63 (Hav6N?).<sup>1</sup> Similarly, no HI antibodies to *Mycoplasma gallisepticum* were detected. Many sera, how-

ever, inhibited the haemagglutinins of the three virus isolates at titres varying from 1:16 to 1:256. Similarity in titre endpoints for a given serum with the three isolates indicated their antigenic identity.

#### Pathogenicity of turkey/Ontario/6118/67 virus

Chickens (4-6 weeks old) and turkeys (4 weeks old) supported intranasal and intraperitoneal infection with isolate 6118 (undiluted allantoic fluid from the third egg passage) without apparent harm. All birds developed HI antibodies to the virus, but in no instance did the titre exceed 1:100.

#### Identification of the virus and antigenic relationship to influenzaviruses of other animal species

Antigenic studies of RNP and surface antigens were carried out independently in three laboratories (Guelph, Ontario; Prague, Czechoslovakia; and

<sup>1</sup> The question mark in the antigenic code indicates that the particular antigen has not yet been determined.

Table 3. Results of neuraminidase inhibition tests with turkey/Ontario 10/6118/68 virus

Neuraminidase subtype	Hyperimmune serum <sup>a</sup>	NI titre with homologous virus <sup>b</sup>	NI titre with turkey/Ontario/6118/68 virus
N1	chicken/Scotland/59	500	<10
	duck/Germany/210/67	>1 000	<10
	duck/Germany/1868/68	300	<10
	duck/Pennsylvania/486/69	500	<10
	swine/Cambridge/39	>1 000	<10
N2	turkey/Massachusetts/65	300	<10
	turkey/Wisconsin/66	>1 000	<10
Neq1	FPV/Dutch/27	300	<10
	N/Germany/49	>1 000	<10
	equine/Prague/56	500	<10
Neq2	turkey/Canada/63	>1 000	<10
	quail/Italy/1117/65	500	<10
	duck/Ukraine/1/63	200	<10
	equine/Miami/63	500	<10
Nav1	duck/England/56	>1 000	<10
	duck/Czech/56	>1 000	<10
	duck/England/62	300	<10
Nav2	tern/South Africa/61	1 000	<10
Nav3	turkey/England/63	300	<10
Nav4	turkey/Ontario/6118/67 <sup>c</sup>	—	800
Human influenza A viruses			
N1	A/BEL	>1 000	<10
N1	A/PR8	300	<10
N1	A/FM1	300	<10
N2	A/Singapore/1/57	>1 000	<10
N2	A/Hong Kong/1/68	>1 000	<10
—	Parainfluenza/turkey/Ontario/6661/67	100	<10
—	Parainfluenza/turkey/Wisconsin/68	100	<10

<sup>a</sup> Turkey/Ontario/6118/68 enzyme relationship was tested in two separate tests with hyperimmune rabbit and rat sera.

<sup>b</sup> Serum dilution inhibiting 50% of neuraminidase activity in tests with 1–2 units of neuraminidase.

<sup>c</sup> Antiserum prepared against turkey/Ontario/6118 failed to inhibit the neuraminidase activity of other viruses used in the tests when used at 1:10 dilution.

Members of each group of viruses (1–6) contain immunologically related neuraminidase.

London, England). The influenza A group antigen in virus 6118 was ascertained by the group-specific complement fixation test (Lief et al., 1958) and the agar-gel double diffusion test (Beard, 1970). The compiled results of comparative haemagglutination inhibition (WHO Expert Committee on Influenza Respiratory Virus Diseases, 1959) and neuraminidase inhibition (Webster & Pereira, 1968; Schild & Newman, 1969) tests are given in Tables 1, 2, and 3, respectively. The test strains included representatives of known human and animal influenzaviruses, yet none of these cross-reacted in the two tests with turkey/Ontario/6118/67 virus.

#### DISCUSSION

Avian influenza has been recognized in North America since 1963, and has recurred annually in sporadic farm outbreaks. Turkeys and ducks, but not chickens, have been found to be naturally infected. The causative viruses fall into those groups that have haemagglutinin subtypes Hav4, Hav5, and Hav6, groups which have also been found in Europe and Africa, and which share some of the antigenic components of the virus envelope with mammalian type A influenzaviruses (Tumová & Pereira, 1968; Pereira, 1969; Schild et al., 1969; Tumová & Easterday, 1969; Schild & Newman, 1969; Webster & Pereira, 1968). Turkey/Ontario/6118 virus is different from the others: it shares the group A antigen but none of the surface antigens with the other influenza A viruses with which it was compared.

The farm on which the outbreak occurred was isolated, but was affiliated with a turkey hatchery where influenza outbreaks caused by the viruses turkey/Ontario 3724/63 (Hav6N?) and turkey/Ontario 5265/67 (Hav5N?) had previously been diagnosed in the breeding stock. Furthermore, 3 months after the 6118 outbreak in the hens, an avian influenza-virus turkey/Ontario 6828/67 (Hav5N?) was isolated from diseased males that had escaped the earlier disease episode, and 1 year later a typical swine influenzavirus (turkey/Ontario 4885/67) was isolated from a new flock of turkeys that had replaced the previous batch after complete depopulation and disinfection of the premises. The infection with a virus having Hav5 haemagglutinin was probably acquired through contact with the hatchery. The most likely source of the swine virus infection was the neighbouring farm (about 1.6 km away) where the pigs were suffering at the time from an undiagnosed respiratory ailment; personnel from this farm formed part of the insemin-

nation crew at the turkey farm. These viruses were included in the serological analysis of virus 6118, but yielded no clue as to the origin and evolution of this new subtype. During the three years following the outbreak, no HI antibodies to virus 6118 were detect-

ed in replacement turkeys on this farm, nor was the infection found elsewhere.

It is proposed that turkey/Ontario/6118/67 virus should be regarded as a new avian subtype and antigenically designated Hav8Nav4.

### ACKNOWLEDGEMENTS

The case was referred to us, and clinical data were supplied, by Dr A. E. Ferguson, Ontario Veterinary College, Guelph. Financial assistance was provided in part by the Ontario Department of Agriculture and Food.

### RÉSUMÉ

#### UN NOUVEAU SOUS-TYPE DE VIRUS GRIPPAL DE TYPE A ISOLÉ CHEZ DES DINDONS

Un nouveau sous-type de virus A de la grippe aviaire a été identifié en janvier 1967 lors d'une épizootie dans un élevage de dindons de l'Ontario (Canada). La maladie a atteint au total 2500 dindes et entraîné la mort de 65 d'entre elles. Le virus a été isolé à partir de tissus pulmonaires et trachéaux prélevés sur trois animaux morts. Les sérums d'oiseaux convalescents renfermaient des anticorps dirigés contre le virus isolé au cours de l'épizootie, mais sans action contre d'autres virus de la grippe aviaire, le virus de la maladie de New-

castle, le myxovirus Yucaipa et *Mycoplasma gallisepticum*.

La souche isolée, appelée A/turkey/Ontario/6118/67, contenait la ribonucléine des virus grippaux A. Son hémagglutinine et sa neuraminidase étaient antigéniquement différentes des antigènes d'enveloppe d'autres virus grippaux isolés chez des oiseaux, des chevaux, des porcs ou chez l'homme. On propose de considérer le virus turkey/Ontario/6118/67 comme un nouveau sous-type des virus A de la grippe aviaire.

### REFERENCES

- Beard, C. W. (1970) *Avian Dis.*, **14**, 337-341  
 Easterday, B. C. & Tumová, B. (1972) *Avian influenza*.  
 In: Hofstad M. J., ed., *Diseases of poultry*, Ames, Iowa University Press  
 Lief, F. S. et al. (1958) *J. Immunol.*, **85**, 53-65  
 Pereira, H. G. (1969) *Proc. roy. Soc. Med.*, **62**, 43-44  
 Pereira, H. G. et al. (1964) *Bull. Wld Hlth Org.*, **35**, 799-802  
 Schild, G. C. et al. (1969) *Nature (Lond.)*, **222**, 1299-1301  
 Schild, G. C. & Newman, R. W. (1969) *Bull. Wld Hlth Org.*, **41**, 437-445  
 Tumová, B. & Pereira, H. G. (1968) *Bull. Wld Hlth Org.*, **38**, 415-420  
 Tumová, B. & Easterday, B. C. (1969) *Bull. Wld Hlth Org.*, **41**, 429-435  
 Webster, R. G. & Pereira, H. G. (1968) *J. gen. Virol.*, **3**, 201-208  
 WHO Expert Committee on Respiratory Virus Diseases (1959) *Wld Hlth Org. tech. Rep. Ser. No. 170*, p. 59