Myxovirus Influenza A Isolated from Ducklings

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ABSTRACT

A strain of haemagglutinating virus isolated by Walker and Bannister in 1953 and identified by them as "Filterable Agent" of ducks has been examined. The evidence suggests that although belonging to the Myxovirus Influenza Type A group, it possesses specific strain properties.

In 1953 Walker and Bannister[1] isolated a "Filterable Agent" from ducklings that had been involved in an outbreak in which the mortality was approximately 20%. Other birds in direct or indirect contact remained healthy. Because the symptoms resembled Newcastle Disease which was a very live issue in Canada at the time, an attempt was made to isolate the causative virus. This resulted in ruling out NCD but demonstrating the presence of what the authors termed a "Filterable Agent". Animals and birds of various ages were inoculated with it by several routes but none including ducklings showed evidence of infection.

At the time attempts to propagate influenza PR8 and NCD in the bovine mammary gland were planned. Because the filterable agent produced haemagglutination of fowl erythrocytes, it also was included. All three appeared to propagate in the gland[2]. Another trial was conducted using PR8 and NCD viruses. It was clearly demonstrated that propagation had taken place. No further outbreaks having occurred and the "Filterable Agent" appearing of little importance led to it being stored away without further study.

Having recently again taken up the problem of the propagation of viruses in the mammary gland and especially of studying serologically the antibody provoked, brought the filterable agent to mind. Fortunately, the strain had been stored in the deep freeze at the Animal Disease Research Institute. This was very courteously made available to the authors and was used in some serological work in which several viruses were included. This led to the identification of the filterable agent.

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Propagation and Tentative Identification of Agent

The material received from the ADRI was contained in frozen allantoic fluid. It was passaged once in 10 day old chick embryos, using the CA route. After 48 hours the allantoic fluid was harvested and tested. It gave an EID_{50} of 10^{-7}.

The CA membranes of these eggs were pooled, all fluids removed and the membranes suspended in an equal volume of N/NaCl solution; these were placed in a Waring Blender and ground. The brei was then centrifuged for 20 minutes at 2500 rpm, the fluid removed and stored at 4°C for 3 days. The fluid was then centrifuged at 1500 rpm for 15 minutes, the supernatant removed and centrifuged at 35,000 rpm for 90 minutes. The supernatant was removed from the precipitate and employed as antigen. Using the Filterable Agent antigen described and standard Influenza A PR8 serum, a complement fixation test was carried out resulting in a positive reaction indicating that the virus in question likely belongs to Influenza Type A.

Further Investigation of the Strain

Assuming the virus belonged to Type A, it was then decided to determine the relationship, if any, to some other members. Through the kindness of Dr. B. C. Easterday the following strains were secured, Type A Duck England 56, Duck Czech and Turkey Wisconsin.

Each of these was passaged once in 10 day chick embryo by the CA route, the allantoic fluids harvested in 48 hours and stored at —80°C.

The Filterable Agent, Turkey Wisconsin and Duck England were each inoculated twice intramuscularly into male

TABLE I. HAI and Neutralization Tests Anti Sera (Treated and Untreated)

Viruses and Types	Turkey (Wisconsin)			Duck (England)			F.A. Duck (Canada)		
	HAI Treated	HAI Untreated	Neut.	HAI Treated	HAI Untreated	Neut.	HAI Treated	HAI Untreated	Neut.
A_0 Anatis (Eng) 56	—	—		1:320	1:640	1:400	—	—	—
A_0 Anatis (Czech)	—	—		—	—		—	—	—
A_0 Turkey (Wisc)	1:160	1:640		—	—	—	—	—	—
A_0 Porci	—	—		—	—	—	—	—	—
A_0 PR8	—	—		—	—	—	—	—	—
A^0 WS	—	—		—	—		—	—	—
A_1 FMI	—	1:20		—	—	—	—	1:20	—
A_1 Weiss	—	—		—	—		—	—	—
A_1 Melbourne	—	—		—	—		—	—	
A_1 Talmey	—	—		—	—		—	—	—
A_2 Singapore	—	1:40		—	1:40		—	1:40	
A_2 Can/57	—	1:20		—	1:20		—	1:20	
B Taiwan	—	—		—	—		—	—	
B Lee	—	—		—			—	—	
B (Gr. Lakes)	—	—		—	—		—	—	
F. A. Duck (Canada)	—	—		—	—	—	1:160	1:320	1:800

fowl allowing an interval of 6 days. Twelve days after the last inoculation the fowl were bled and serum separated from the clot. This was divided into two lots. One was treated with potassium periodate, the other left untreated.

With the three antisera a number of strains of Influenza A of different host origins and three strains of Influenza B were tested by the HAI, neutralization or both methods. The results are shown on *Table I.*

Hamsters were given the Filterable Agent by the intra-nasal route. In 21 days they were bled and the serum harvested. Using this antiserum a number of strains of influenza were examined by complement fixation. Details are given in *Table II.*

Antisera of four influenza strains were examined by complement fixation using the Filterable Agent antigen already described. The results are shown in *Table III.*

The information contained in Tables I, II and III appears to leave little doubt regarding the nature of the Filterable Agent. Turkey, Duck (England) and F.A. Duck (Canada) antisera each gave well marked reactions to their respective antigens but no significant reactions to other strains in the HAI and neutralization tests.

Complement fixation tests using F.A. Duck (Canada) anti-serum and the following antigens — a number of strains of Type A, one strain of Type B and Myxovirus parotidis — gave some degree of reaction with all Type A strains, the majority of fowl strains showing a somewhat higher titre. No reaction to Type B or Myxovirus parotidis was evident.

When F.A. Duck (Canada) soluble membrane antigen was employed against antiserum Influenza A, PR8 and Porci a positive reaction resulted while the same antigen used against Influenza B and C gave no reaction.

On the basis of the results of the several trials contained in Tables I, II and III it

TABLE II. Complement Fixation Antiserum F. A. Duck (Canada) and Several Myxovirus Strains

Antisera Hamster F. A. Duck (Canada)	Antigen	Results
F. A. Duck (Canada)	F. A. Duck (Canada)	+ 1:128
"	A PR8	+ 1:64
"	A FMI	+ 1:64
"	A_2 Singapore	+ 1:64
"	A Porci	+ 1:32
"	A Duck Eng 56	+ 1:32
"	A Duck Czech	+ 1:128
"	A Turkey Wisc.	+ 1:128
"	B-Lee	—
"	C Myxo. Parotidis	—
"	Normal Membrane	—
"	N/Saline	—

TABLE III. Complement Fixation F. A. Duck (Canada) Soluble Membrane Antigen and Several Antisera

Anti-Sera	Antigen F. A. Duck (Canada)	Results
A_0 PR8	F. A. Duck (Canada)	+
A_0 Porci.....	F. A. Duck (Canada)	+
B-Lee.....	F. A. Duck (Canada)	−
Infl. C.....	F. A. Duck (Canada)	−

would appear that the Filterable Agent isolated by Walker and Bannister belongs to Influenza Type A.

Comments

There are few areas in the field of infectious diseases more interesting than that of influenza. This is in part the result of a broader approach to the problem in recent years in which the animal kingdom as a whole rather than man alone is now considered. The work of the World Influenza Centre, National Institute of Medical Research, London, England under H. G. Pereira is an example of this broad approach.

Long before the days of virology there was a suspicion that human and equine influenza shared a common cause. Many years ago several veterinary clinicians drew attention to simultaneous outbreaks of influenza in man and horse[3]. During the great equine outbreak of 1872 the New York Health Department attempted to collect information on the nature of the equine outbreak in the hopes it might throw light on epidemics in man. A commission was appointed whose most prominent member was Judson. In the Committee's report the conclusion is unequivocally drawn that Epizootic influenza is the counterpart of Epidemic influenza[4]. Following the pandemic of 1918 a very interesting paper by Bemelmans[5] also drew attention to this question.

The explosive outbreaks have much in common. Two examples illustrate this curious feature of developing a local focus of infection which quickly spreads to involve large areas. The great outbreak of equine influenza, 1872, and the pandemic in man of 1918 are called to mind.

The former apparently arose in Toronto[6], spread north to the Huron-Georgian Bay area and south to the United States. It quickly overran Canada from Ontario to the Atlantic area excepting Prince Edward Island. The spread in the U.S. was equally rapid until it involved the majority of the States of the Union. From the Western it spread to the Eastern hemisphere and finally involved many countries[6]. The great pandemic of human influenza during the first world war apparently commenced in 1918 in Western France; it spread quickly throughout the world causing at least 20 million deaths. Rhodes and Van Rooyen[7] suggest that the intermingling of soldiers may have been conducive to an exchange of respiratory flora leading to the development of a strain of singular virulence capable of spreading rapidly. It should be kept in mind that some of the troops were in close contact with the many horses that also were attacked with clinical influenza.

Work in recent years indicates that influenza viruses having antigenic relationship have a very extensive host range. In keeping with this, it was thought desirable even at this late date to draw attention to the nature of the "Filterable Agent" of ducks isolated by Walker and Bannister[1].

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REFERENCES

1. **WALKER, R. V. L. and BANNISTER, G. L.** A filterable agent of ducks. Can. Jour. Comp. Med. and V. Sc. 17: 248, 1953.
2. **MITCHELL, CHAS. A., WALKER, R. V. L. and BANNISTER, G.L.** Preliminary experiments relating to the propagation of viruses in the bovine mammary gland. Can. Jour. Comp. Med. 17: 97, 1953.
3. **FLEMING, G.** Animal plagues their history, nature and prevention.
4. **JUDSON, A. B.** Epizootic infection of horses counterpart of epidemic infection of man. Veterinarian Vol. XLVII — L, p. 588, 1874.
5. **BEMELMANS, E.** A comparative study of human grippe and contagious pleura pneumonia of the horse (equine grippe). Jour. Am. Vet. Med. Assoc. 15: 52-69, 1922-1923.
6. **JUDSON, A. B.** The origin and progress of the epizootic among horses in 1872. Veterinarian XLVII — L, p. 492, 1874.
7. **RHODES and VAN ROOYEN.** Text Book of Virology, 3rd. Edition.