the Biological Standards Laboratory, Hampstead, who sent us serum specimens; and the Distillers Company Ltd., Great Burgh, Epsom, who generously gave one of us (E. J. C. K.) supplies of "cardice."

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HUMAN INFLUENZA VIRUSES IN DOMESTICATED ANIMALS

BY

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During the 1957 pandemic of Asian influenza W.H.O. initiated a study into the possible role of animals in the epidemiology of influenza (Kaplan and Payne, 1959). More than 20 countries participated, and the findings suggested that A2 viruses can cause inapparent infection in horses and swine, which were the only animals tested. In addition, serological evidence of A/equi influenza virus was discovered in countries which had not previously been aware of its presence. The results from the material tested in Ireland at that time were equivocal and no conclusions could properly be drawn from them.

The present study was undertaken in an attempt to elucidate the Irish findings and to attempt to enlarge or confirm those from other countries. Sera were collected at intervals from a variety of animals in Ireland from the early months of 1960 until the summer of 1961. During this time a major influenza epidemic occurred in January, 1961 (Meenan and Boyd, 1962).

Materials and Methods

Antigens.—The following strains of influenza virus were used: PR8; FM-1; A2/Asia/57; A2/Eire/8/61, isolated in Dublin during the 1961 epidemic; A2/Asia/57(Ashton) and A2/Singapore/W/1/57, inhibitor insensitive strains forwarded to us by Professor Belyavin; A/equi/Praha/56 and A/swine/Shope/15/33, both forwarded by Sir Christopher Andrewes.

Sera.—Horse sera were obtained from two abattoirs in and near Dublin: a few were received from animals showing signs of respiratory infection. *Swine sera* were obtained from a Dublin meat-processing plant. *Cattle sera* were obtained from a Dublin abattoir. *Poultry (hen) sera* were obtained from a Dublin chicken-processing factory. *Cat and dog sera* were obtained from veterinary dispensaries, and were from animals which were being destroyed. All sera were stored at −20° C. before use.

Complement-fixation (C.F.) Tests.—The method used was that of the W.H.O. (1953) Expert Committee on Respiratory Virus Diseases. PR8 virus was used for the preparation of soluble antigen.

Haemagglutination Inhibition (H.I.) Tests. — The method used was that of the W.H.O. (1953) Expert Committee on Respiratory Virus Diseases, except that the serum–virus mixtures were allowed to stand at room temperature for 30 minutes before the addition of red cells. Fowl red cells were used with W.H.O. pattern plastic plates.

The sera were treated as follows: 0.1 ml. of serum was heated for 30 minutes at 56° C., after which 0.3 ml. of *M*/90 potassium periodate was added and the mixture allowed to stand at room temperature for 30 minutes. Then 0.3 ml. of 1% glycerol-saline was added. A proportion of the horse sera was tested after similar treatment but without pre-heating. The results of the test were read by the pattern method after standing for one hour at room temperature.

Serum controls were included in each test, and sera showing direct agglutination of fowl red cells were omitted from the results.

Virus Isolation.—Throat and nasal swabs from animals with respiratory diseases were stored at −20° C. in 3 ml. of Hanks's lactalbumin containing 10% calf serum with added penicillin and streptomycin. Lung tissues from suspicious fatal cases were frozen and thawed quickly three times, centrifuged at 3,000 r.p.m. for 15 minutes, and, if necessary, stored at −20° C. before inoculation. All specimens were inoculated amniotically into 13- or 14-day chick embryos, and also into monkey-kidney and HeLa cell tissue cultures. The eggs were examined for evidence of growth of haemagglutinating viruses; HeLa cells for cytopathogenic effect; and the monkey-kidney cultures for cytopathogenicity, haemadsorption, and haemagglutination. All specimens were given a further blind passage.

Results of Virus Isolation

The results are shown in Table I. No viruses were recovered from any of the material examined.

TABLE I.—*Results of Virus Isolation Studies*

Animal	1960	1961	Viruses Isolated
Cattle	10	0	0
Sheep	6	0	0
Poultry	9	0	0
Horse	40	14	0
Dog	15	1	0
Swine	29	0	0

Serological Results

C.F. Tests

Of the 2,157 sera examined, 369 were collected after the January, 1961, epidemic. The results are shown in Table II. It will be seen that with the exception of two

TABLE II.—*Results of Complement-fixation Tests*

Animal	No. Tested	Dilution	Positive
Sera Collected before Epidemic			
Horse	217	1 : 4 1 : 8	2 (at 1 : 4)
Dog	22	1 : 4	0
Cattle	229	1 : 4	0
Swine	512	1 : 4	0
Poultry	808	1 : 4/1 : 8	0
Sera Collected after Epidemic			
Horse	147	1 : 4	0
Cattle	99	1 : 4	0
Swine	96	1 : 4	0
Poultry	27	1 : 4	0

horse sera, both collected before the epidemic and positive to a low titre, C.F. tests were uniformly negative and did not suggest that influenza viruses circulated to any extent in animals during the period covered by this study. The H.I. titres for these two sera against A/equi were 1:640 and 1:160 respectively.

H.I. Tests

Swine.—In all, 454 were examined for evidence of infection with Shope virus, and were completely negative at a dilution of 1/20 or greater. It would seem, therefore, that this virus is not established in the swine population in Ireland. In addition, 59 sera were tested using A2/Asia/57 as antigen. These also were negative, so that, taking these results in conjunction with the negative complement fixation tests, there would seem to be no evidence for a reservoir of A2 viruses among swine in this country. At the time of the A2 epidemic in 1957 lung tissues were obtained from 30 pigs showing pneumonic lesions at the time of slaughter. These were examined, using chick embryos, for evidence of A2 infection. No viruses were isolated (Meenan, unpublished observations).

Cattle.—The results given in Tables II and III suggest that there is no reason to implicate cattle in the epidemiology of influenza, nor that even widespread human infection spreads to them.

Poultry.—These results are more difficult to interpret than are those for swine or cattle, but, taken together with the C.F. findings, they suggest that the small number of positives against A2/Asia/57 are in fact due to non-specific inhibition (Tables II and III). A large proportion of the fowl sera tested contained a considerable amount of lipid, so that it was necessary to centrifuge the sera and decant this before treating it with periodate. The presence of this rather than of true antibody seems the most likely explanation of the positive findings.

Dogs and Cats.—The sera from these were all from domestic pets and were collected at the time they were being destroyed. An intravenous anaesthetic was used in all cases. Many of the specimens were badly haemolysed on receipt and clearly contained a substantial amount of the anaesthetic. This is the more unfortunate as dogs and cats might be liable to infection in a household where there are several cases of influenza. The sera were all collected prior to the epidemic (Tables II and III).

Horses.—The results of the H.I. tests require detailed consideration as they depend on the antigen used. As already stated, almost all of the horse sera examined came from abattoirs and practically all of these animals were at least 12 years old. In view of the findings reported below and those reported by others (Kaplan and Payne, 1959; Schaeffer and Robinson, 1961) it was of interest to determine whether any evidence of infection with the older influenza A viruses could be obtained. The results were completely negative (Table III). On the other hand, the results suggest that A/equi/Praha virus may be present among horses in Ireland, although no outbreak due to it could be discovered during this study and no strain of it has yet been isolated.

TABLE III.—*Results of H.I. Tests*

Antigen	No.	Positive at: 1/20	1/40	1/80	1/160	1/320	1/640	1/1,280	1/2,560	1/5,120
Cattle Sera										
A2/Asia/57	60	0	0	0	0	0	0	0	0	0
A2/Asia/57 (Ashton) ..	141	0	0	0	0	0	0	0	0	0
A/equi/Praha	101	0	0	0	0	0	0	0	0	0
A2/Eire/8/61	101	0	0	0	0	0	0	0	0	0
Poultry Sera										
A2/Asia/57	58	3	0	3	0	0	0	0	0	0
A2/Asia/57 (Ashton) ..	10	0	0	0	0	0	0	0	0	0
Dog Sera										
A2/Asia/57	45	4	2	2	0	0	1	1	3	0
A2/Asia/57 (Ashton) ..	81	0	0	0	0	0	0	0	0	0
Cat Sera										
A2/Asia/57	19	0	1	0	0	0	2	0	1	0
A2/Asia/57 (Ashton) ..	20	0	0	0	0	0	0	0	0	0
Horse Sera										
PR8	106	0	0	0	0	0	0	0	0	0
FM-1	109	0	0	0	0	0	0	0	0	0
A/equi/Praha:										
Before epidemic ..	451	13	12	4	2	1	1	0	0	0
After ,, ..	410	14	5	3	0	0	0	0	0	0

A_2 Viruses

Table IV shows the titres obtained when a standard A2/Asia/57 antiserum (Phillips-Roxane, obtained through W.H.O.) was tested against the different strains of A2 viruses used in the study and against A/equi/Praha/56.

TABLE IV.—*Results of H.I. Tests with Standard Antiserum and with Untreated Horse Serum*

Virus	Titre of Inhibition: Standard Antiserum	Untreated Horse Serum
A2/Asia/57	640	>5,120
A2/Eire/8/61	320	>5,120
A2/Asia/57 (Ashton) ..	40	<10
A2/Singapore/W/1/57 ..	320	<10
A/equi/Praha/56 ..	<10	<10

The sensitivity of the same strains to non-specific inhibitor is also given in Table IV, derived from a typical experiment using a horse serum which had received no pretreatment. From this it can be seen that the strains A2/Asia/57 and A2/Eire/8/61 were exceedingly sensitive to inhibitor, while the other A2 strains used were insensitive. However, A2/Asia/57 (Ashton) was also relatively insensitive to antibody. The results obtained using these four strains with pretreatment of the horse sera are summarized in Table V.

It seems clear from these results that the strain of virus used in H.I. tests for evidence of infection among horses with A2 viruses is of fundamental importance, and that treatment with periodate or even heat followed by periodate may not be sufficient to remove non-specific inhibitor.

TABLE V.—*Results of H.I. Tests on Horse Sera Using A2 Strains*

Antigen	Pre-Epidemic	Post-Epidemic	Heat + KIO_4	KIO_4 Only	No. Tested	No. Positive at: 1/20	1/40	1/80	1/160	1/320	1/640	1/1,280	1/2,560	1/5,120
A2/Asia/57	+		+		446	65	51	44	11	11	11	6	1	1
A2/Asia/57	+			+	171	21	19	9	6	3	3	3	0	0
A2/Eire/8/61 ..	+			+	260	54	44	33	14	8	2	1	1	1
A2/Eire/8/61 ..		+	+		144	34	17	4	0	0	0	1	0	2
A2/Asia/57 (Ashton)	+			+	123	0	0	0	0	0	0	0	0	0
A2/Asia/57 (Ashton)	+		+		187	0	0	0	0	0	0	0	0	0
A2/Asia/57 (Ashton)		+	+		121	0	0	0	0	0	0	0	0	0
A2/Sing./W/1/57 ..	+			+	204	0	0	0	0	0	0	0	0	0
A2/Sing./W/1/57 ..		+		+	206	1	0	0	0	0	0	0	0	0

Discussion

This study may be regarded as comparable with that of Kaplan and Payne (1959) in so far as it was initiated before an epidemic caused by A2 virus and continued after it. It so happened that in Ireland the 1961 epidemic was more widespread and much more severe than that of 1957. Meenan and Boyd (1962) have described this epidemic, which was imported from the Birmingham region of England by people returning home for the Christmas holidays. With this fortunately clear-cut epidemiological background an opportunity arose to see how far, if at all, widespread human infection might be reflected among domesticated animals. It might reasonably have been expected also that if a reservoir of human influenza virus had existed among these animals its size would have been very much enhanced during the epidemic.

Ireland is predominantly an agricultural country and the importation of animals is on a very small scale. That this may be of importance is reflected in the fact that Q fever, for example, has not been detected in any part of Ireland. The presence or absence of an animal reservoir in Ireland, which is an island and relatively isolated, is therefore not necessarily indicative of the position on the continents of Europe and Asia or of North America. On the other hand, the discussion of our findings may have a bearing on the validity of certain aspects of the 1957–8 study.

From the serological results it seems that A/equi virus may be present in Ireland although not to any great extent. The two positive C.F. tests in horses, albeit of low titre, may be confirmatory of this. No epizootic due to it has yet been recognized, and no outbreaks of acute respiratory infection among horses were reported during this study. Antibody levels to this virus among horses were not affected by the epidemic, although, were there any widespread transmission of human infection to horses during it a boosting effect on previous levels might have taken place. In fact, however, the antibody levels to A/equi were, if anything, slightly lower after the epidemic, and no positive sera were detected in C.F. tests after it, all of which is against any spread of infection with influenza viruses from man to horse.

The position regarding A2 infection among horses seems at first sight more complicated. Table V shows that a high proportion of positive H.I. tests was obtained with both A2/Asia/57 and A2/Eire/8/61. On purely epidemiological grounds it is difficult to understand how such apparently widespread infection could have occurred so silently. It may be noted that in the two human epidemics caused by A2 viruses there has been no doubt whatsoever of their presence. Furthermore, there was no significant difference between the proportion of positive horse sera collected before and after the epidemic. This finding also would be surprising if these viruses spread from man to horses. Antibody levels were significantly higher among the human population after the 1961 epidemic (Meenan and Boyd, 1962).

Kaplan and Payne (1959), on the basis of the serological information supplied to them by the participating laboratories, reached the conclusion that the A2 strains may cause natural infection in horses, although they noted that both natural and experimental infections were entirely silent. Schaeffer and Robinson (1961) found 51 positives in examining 228 paired sera collected before and after an epidemic of human influenza. They were not impressed by the specificity of these results, attributing them to non-specific inhibition.

Both the A2/Asia/57 and A2/Eire/8/61 strains are extremely sensitive to non-specific inhibitor—so sensitive indeed that its complete removal is a matter of considerable difficulty—while the A2/Asia/57 (Ashton) and A2/Singapore/W/1/57 strains are both resistant to it. The Ashton strain was also somewhat resistant to specific antibody in our hands, but the A2/Singapore/W/1/57 was sensitive (Table IV). When the results obtained with these two strains are analysed it is seen that no positive sera were detected using Ashton and only one doubtful (to a low titre) among 410 sera tested against A2/Singapore/W/1/57. The reasonable conclusion seems to be that the results obtained with the other A2 strains, both by ourselves and by other workers, could have been due to non-specific inhibitor even after the most rigorous pretreatment to remove it. Such a conclusion fits in also with the epidemiological findings, so that the evidence for spread of A2 viruses to horses is very tenuous. Certainly in Ireland, at least, it seems that it does not occur.

As for swine, it appears that the Shope virus is not present among them in Ireland and there is as yet no evidence that A2 infection has spread to them. Dogs and cats are obviously exposed to infection during epidemics, but here again the results using the Ashton strain were completely negative. There were also considerable difficulties in collecting the sera (which were tested before the epidemic), and the presence of large quantities of anaesthetic may help to account for the positive results against A2/Asia/57. Further study of these animals might be rewarding, but even confirmed infection among them would not necessarily include them in an infectious cycle involving man. They are so exposed to human infection that it would seem right to regard infection among them as merely a reflection of human influenza until the contrary is proved.

In summary, there is no evidence for the existence of a reservoir of A2 viruses among domesticated animals in Ireland, and there is no evidence for spread of infection to them during a major A2 epidemic.

Summary

The results of isolation and serological tests for human A2 viruses among swine, cattle, poultry, dogs, cats, and horses before and after an epidemic in Ireland are presented.

There was no evidence for the presence of either Shope or A2 virus among swine. The results for cattle were also uniformly negative.

Cats, dogs, and poultry show a small number of positives with A2/Asia/57 virus. Reasons are given for regarding the positive results as non-specific.

Among horses A/equi/Praha virus, or one closely related to it, may be present, but no epizootic due to it has been described and no strains of it have been isolated. Positive results with A2/Asia/57 and A2/Eire/8/61 were obtained, but none with A2/Asia/57 (Ashton) or A2/Singapore/W/1/57. Technical and epidemiological reasons are given for regarding the positive results as non-specific.

It is concluded that there is no reservoir of A2 viruses in animals in Ireland, and that no spread to them occurred during the January, 1961 epidemic.

This investigation was supported by Grant No. E-2733 from the National Institutes of Health, American Public Health Service.

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ABO BLOOD GROUPS AND ACUTE RESPIRATORY VIRUS DISEASE

BY

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Acute respiratory virus infections are responsible for many deaths before and during reproductive life and some effect might therefore be expected on natural selection. It seemed possible that the inheritance of any natural resistance to these diseases would be related to blood groups or other inherited characteristics.

Surveys of acute respiratory illness have been carried out jointly by the Directorate of Hygiene and Research of the Royal Air Force and the Public Health Laboratory Service since 1954. In the course of this work a large number of men admitted to sick quarters with a respiratory illness were investigated virologically and the causal infection was identified. Since 1956 all recruits to the Royal Air Force have been tested for blood group on entry, and this, together with such other information as place of birth, colour of eyes, hair, and skin, and colour perception, has been recorded in their personal documents. The means were thus available for an investigation of this problem and a unique opportunity was afforded by the appearance in 1957 of the influenza A2 group of viruses which the population had not encountered previously. This could not be said for most of the other virus groups—adenovirus in particular—to which the men had been exposed for many years.

Materials and Methods

Information was taken from the records held by the Air Ministry of just over 2,000 persons admitted since 1956 to station sick quarters with a respiratory illness associated with one or more identified virus infections. Only diagnoses based on virus isolation or a fourfold or greater rise in antibody titre were accepted. The great majority of the patients were new recruits, mostly 18–20 years of age. Records were incomplete in about 100 patients and 52 had been born outside the British Isles, leaving 1,875 available for study.

The distribution of blood groups to serve as a control was kindly supplied by Dr. A. C. Kopéc, of the Nuffield Blood Group Centre, from a sample of 47,108 R.A.F. recruits, also born in the British Isles, who had been tested during the period of our own surveys, 1956–61. The results of this sample, divided into three regional groups according to the recruit's place of birth, are shown in Table I. Counties south of a line from the Wash to the Severn were included in region 1, the remaining counties of England and Wales and four in South-west Scotland in region 2, and the rest of Scotland and Ireland in region 3.

TABLE I.—*Distribution of Blood Groups in* 47,108 *R.A.F. Recruits*, 1956–61*

Place of Birth	No. Tested	Group O	Group A	Group B	Group AB
Region 1 (S. England)	21,134	9,244 (43·7%)	9,556 (45·2%)	1,700 (8·0%)	634 (3·0%)
,, 2 (N. England, Wales, and S.W. Scotland)	20,043	9,456 (47·2%)	8,265 (41·2%)	1,725 (8·6%)	597 (3·0%)
,, 3 (rest of the British Isles)	5,931	2,994 (50·5%)	2,095 (35·3%)	646 (10·9%)	196 (3·3%)

* Supplied by Dr. A. C. Kopéc, of the Nuffield Blood Group Centre.

Results

The distribution of blood groups in 1,685 patients in five main diagnostic categories is shown in Table II, which does not include 152 patients with evidence of

TABLE II.—*Distribution of Blood Groups in* 1,685 *Patients with Selected Respiratory Virus Diseases*

Infection	Region of Birth	No. of Patients	Group O	Group A	Group B	Group AB
Influenza A1	1	69	33	31	4	1
	2	45	22	17	4	2
	3	15	8	5	1	1
Influenza A2	1	313	171	104	31	7
	2	316	167	111	32	6
	3	72	46	22	3	1
Influenza B	1	32	19	9	2	2
	2	25	8	14	2	1
	3	6	2	4	0	0
Adeno-virus	1	351	134	179	29	9
	2	211	86	92	19	14
	3	105	48	43	9	5
Cox-sackie A21	1	52	29	19	3	1
	2	58	29	27	2	0
	3	15	9	5	1	0

*Attached to the Epidemiological Research Laboratory.