Hong Kong A-2 Influenza Virus Infection among Swine during a Human Epidemic in Taiwan

THE role of lower animals in the natural cycle of human strains of influenza virus transmission has been subjected to considerable speculation. With the possible exception of the report of Romvary *et al.*[1], there are few, if any, satisfactorily authenticated cases of human influenza viral strains being isolated from naturally infected animals. That animals could be naturally infected was demonstrated when antibodies against the Asian A-2 strain were found in swine and horses after the 1957 pandemic[2].

Because of an accessible large porcine population on Taiwan (1959 census—3,529,000 pigs; 14,350,000 humans), an influenza surveillance study was recently initiated (June 1969) to determine whether pigs play a part in human infection. The study will continue for at least 12 months; but because of noteworthy results obtained during and immediately after an island-wide outbreak in humans (November 1969–January 1970), we are submitting this interim report.

Sera and nasal swabs are being obtained from apparently healthy 6 month old pigs from several Taiwan slaughterhouses. Sera were tested by haemagglutination-inhibition (HI) test against three antigens: A-2/Japan 305/1957 (A-2/Jap), A-2/Hong Kong/1968 (A-2/HK) and B/Lee/1940 (B/Lee). An HI titre of 1 : 40 or greater is considered positive.

For isolation studies swine nasal swab materials, obtained just before slaughter, were injected into primary monkey kidney tissue culture tubes, incubated, and observed for viral growth by use of the haemadsorption technique. The identity of viral isolates was made by HI test, using reference influenza and parainfluenza antisera. Re-isolation attempts were made in tissue culture and fertile hens' eggs.

Table 1. APPEARANCE OF A-2/HONG KONG 1968 INFLUENZA ANTIBODY IN, AND RECOVERY OF INFLUENZA VIRUS FROM, TAIWAN PIGS

1969–1970	Antibodies against A-2/HK Positives*/tested	Per cent positive	Isolation results†
June–Nov.	5/591	1	0/565
December	2/183	1	1/139
January	48/276	17	11/276
February	92/239	38	1/179

* Haemagglutination-inhibition titre of 1 : 40 or greater. All sera were negative against A-2/Japan 305/1957 and B/Lee/1940.
† All isolates tentatively identified as A-2/Hong Kong. Two identifications confirmed to date (see text).

Results are shown in Table 1. From June to November, 5 of 591 pigs were seropositive; all were at the minimal 1 : 40 endpoint. In December, January and February the seropositives were 2, 48 and 92 respectively. The first viral isolate was obtained in December, followed by 11 in January and 1 in February. In other studies influenza A-2/HK isolates were obtained from humans in November and December 1969 during the epidemic occurring at that time in Taipei.

All sera were specific to the Hong Kong strain. There were no responses above the 1 : 20 level against A-2/Jap or B/Lee. In our tests all viruses were specific to HK antisera. We have re-isolated the virus from twelve of the original thirteen swabs in tissue culture tubes and/or fertile hens' eggs. All isolates have been sent to Dr Walter Dowdle of the WHO International Influenza Center for the Americas, National Communicable Disease Center, Atlanta, Georgia, for definite characterization. He has confirmed the first two isolates tested as being indistinguishable from prototype Hong Kong A-2 viruses. Representative seropositive swine sera have been sent to him for confirmatory laboratory testing, and we have sent him the original nasal swabs for independent re-isolation. These results are pending. It is difficult to assess the significance of these findings at this early date. We are trying to gather data on when the influenza epidemic affected the areas of the pig farms. Preliminary indications are that the infection appeared in man before it appeared in swine. Further speculation on the role of the pig and other mammalian hosts in the epidemic and possible interepidemic cycle of human influenza will have to await subsequent reports.

I thank Mr Norman Chen and Mr G. S. Irving for technical assistance. This study was supported in part by the Bureau of Medicine and Surgery, Navy Department.

W. D. KUNDIN

US Naval Medical Research Unit No. 2,
Taipei, Taiwan, and
Department of Preventative Medicine,
University of Washington,
Seattle, Washington.

Received April 6, 1970.

[1] Romvary, J., Takatsy, Gy., Barb, K., and Farkas, E., *Nature*, **193**, 907 (1962).
[2] Kaplan, M. M., and Payne, A. M.-M., *Bull. Wld Hlth Org.*, **20**, 465 (1959).

Replication of Murine and Feline RNA-containing C-type Viruses in Human Lymphoblastoid Cells

MURINE and feline RNA-containing C-type viruses can replicate in human fibroblast-like cell monolayers with the establishment of virus shedding carrier cultures[1–4]. We describe here the establishment of virus shedding carrier cultures in human lymphoblastoid cell suspensions with a murine sarcoma virus (MSV) and a feline sarcoma virus (FSV).

A virus shedding carrier culture of the Kirsten strain of MSV (Ki-MSV) in human adult fibroblast cells obtained from Dr V. Klement was maintained with Eagle's minimal essential medium (MEM) containing 10 per cent foetal calf serum (FCS) and antibiotics. A strain of FSV obtained from Dr M. Gardner[5] was used to transform beagle embryo fibroblast cells. The transformed cells were cloned and a virus shedding carrier culture was established. These cells were maintained with MEM containing 10 per cent FCS and antibiotics.

Two human lymphoblastoid cell lines (Raji and HR1K) derived from Burkitt lymphomas were maintained with medium RPMI 1640 containing 20 per cent FCS and antibiotics. The Raji cells do not contain Epstein–Barr virus (EBV) particles[6] whereas HR1K cells obtained from Dr J. Grace, jun., do produce varying amounts of EBV particles[7]. The Raji and HR1K cells were passaged by seeding 50 ml. of a cell suspension containing 2×10^5–3×10^5 cells/ml. in C-32 fluid ounce bottles. The cultures were incubated in a horizontal position at 37° C in an atmosphere of air and 3 per cent CO_2. Medium was added after 4 days to bring the volume to 200 ml. By day 7 the cell density had reached a stationary level of $1{\cdot}5 \times 10^6 - 2{\cdot}0 \times 10^6$ cells per ml. with 95 per cent viability as determined by trypan blue exclusion.

The virus containing media from Ki-MSV infected human fibroblast cultures and FSV infected canine cultures were clarified at 10,000*g* for 20 min, and the virus was pelleted at 53,700*g* for 2 h. The pellets were resuspended in medium to give a final concentration of 100 times. Human lymphoblastoid cells (10^7 viable cells) were resuspended in 1·0 ml. of the virus suspension and infected on a magnetic stirrer at 37° C under CO_2 for 2 h. The cells were washed twice and were resuspended in approximately 20 ml. of medium to give a viable cell concentration of 5×10^5 cells/ml. The cultures were incubated and medium was added at intervals to maintain the cell density at a maximum level of approximately