J. Hyg., Camb. (1978), **80**, 415
Printed in Great Britain

Serological evidence of continuing infection of swine in Great Britain with an influenza A virus (H3N2)

By M. S. CHAPMAN, P. H. LAMONT and J. W. HARKNESS
Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey

(*Received* 18 *October* 1977)

SUMMARY

Serum samples collected from swine and cattle in Great Britain at various times between July 1971 and July 1977 were examined by haemagglutination-inhibition or single radial haemolysis methods for evidence of infection with influenza A (H3N2) viruses. A small proportion of swine sera collected in each year reacted in the tests but there was no evidence of infection in cattle. The significance of the findings is discussed, with particular reference to the seasonal fluctuations in the prevalence of antibody in swine observed during the period of the study, and their possible relevance to influenzal events in the human population. None of the sera tested had antibody to swine influenza strains (HSw1N1).

INTRODUCTION

The variant of influenza virus isolated in 1968 by Chang (1969) in Hong Kong spread rapidly to many areas of the world, replacing Asian influenza (antigenically H2N2) as the main cause of epidemic influenza in man. A Hong Kong type virus (H3N2) was isolated from swine in Taiwan in 1969 which together with serological evidence indicated that swine had become infected from man following an epidemic in humans (Kundin, 1970). The Hong Kong virus was first isolated from humans in Great Britain in August 1968 (Roden, 1969) and subsequently, serological evidence was obtained which suggested that the pig population in this country became infected with Hong Kong, or an antigenically similar virus, in the early months of 1970 (Harkness, Schild, Lamont & Brand, 1972). The present report records the results of examinations performed on swine sera collected in later years, and of a search for antibody to influenza A viruses in the cattle population. The aims of the study were to discover whether there was evidence of continuing infection among swine in Great Britain, and whether cattle had become involved in the epidemiology of the virus.

MATERIALS AND METHODS

Influenza viruses

Strains A/Hong Kong/1/68 (H3N2), A/Port Chalmers/73 (H3N2), A/Swine/Wisconsin/66 (HSw1N1) and A/Swine/1976/30 (HSw1N1) used in these studies

were obtained from Dr G. C. Schild, National Institute for Biological Standards and Control, Hampstead, London.

The viruses were cultivated in the allantoic cavity of embryonated hens eggs. Allantoic fluids were harvested after 2 or 3 days incubation, clarified in a bench centrifuge and held either at −20 °C or at 4 °C during studies.

Antisera

Antisera to the A/Hong Kong/68 (H3N2) virus were prepared both in cattle and in pigs by inoculation of formalin-inactivated virus received from Dr G. C. Schild. Antisera to other strains were prepared in guinea pigs by intranasal instillation of live virus; a single dose was sufficient to induce antibody formation.

Haemagglutination-inhibition (HI) tests

These tests were carried out in plastic agglutination trays using 0·2 ml volumes and the standard methods described by the WHO Expert Committee on Respiratory Virus Diseases (1959). To destroy non-specific inhibitors sera were treated with receptor-destroying enzyme (*V. cholerae* filtrate, produced by Wellcome Reagents Ltd, London) overnight at 37 °C followed by heating at 56 °C for 30 min to destroy residual enzyme activity. After absorption with a 10% suspension of chick erythrocytes for 1 h at 37 °C sera were tested against four haemagglutinating units of virus. Virus and serum were incubated for 60 min at room temperature before the addition of a 0·7% suspension (v/v) of chick erythrocytes.

Single radial haemolysis test (SRH)

The test method employed was that described by Schild, Pereira & Chakraverty (1975) with minor modifications. With swine and bovine sera, optimal patterns of lysis were obtained when the concentration of chicken erythrocytes in the gel was adjusted to 15% (v/v). Virus was adsorbed to erythrocytes by mixing equal volumes of a 15% suspension of erythrocytes, and allantoic fluid with a haemagglutination titre of 1/128 or greater. The mixture was incubated at room temperature for 10–15 min before use. Complement (freeze dried guinea pig serum, Wellcome Reagents Ltd, London) was reconstituted with distilled water, and then diluted 1/2 in 0·01 M phosphate-buffered saline pH 7·2. The test gel consisted of 2·6 ml agarose (A. 37, Indubiose), mixed with 0·3 ml of a suspension of the virus-erythrocyte complex, and 0·1 ml of diluted complement. When the gel had set, 2 mm. diameter wells were cut out, and filled with test sera (approximately 5 μl). Plates were incubated at 37 °C in a moist atmosphere for 4–6 h, and the presence or absence of a zone of lysis surrounding the well was recorded for each serum tested.

Virus neutralization tests

Serum neutralization tests with bovine sera were performed with 100 TCID50 of A/Hong Kong/1/68 virus (calf kidney adapted strain) using procedures described previously (Harkness *et al.* 1972).

Sera

Swine sera were collected from individuals in herds experiencing a variety of clinical disease syndromes and were received at the laboratory in conjunction with requests for diagnostic examinations. Samples tested were from herds in various parts of the country and were stored at −20 °C before examination.

Bovine sera examined in HI tests were received between 1968 and 1970 and were collected for the same reasons as the swine sera. Bovine sera examined by the SRH test were from a large number of sera received between November 1974 and July 1975 in connexion with Brucellosis Eradication Schemes. These sera were selected to provide a representative sample on a geographical basis of the cattle population in England and Wales. All bovine sera were also stored at −20 °C before test.

RESULTS

Porcine sera

A total of 1049 swine sera collected during July and August 1971 were examined in the HI test for antibody to A/Hong Kong/1/68 (H3H2) virus. Titres of 1/40 or greater were found in 39 sera (3·7 %), originating from 9 of 32 counties sampled in England. The proportion of sera positive in each county varied from 18/78 for Kent (23 %) to 1/105 for Yorkshire (0·9 %). A further 21 sera had titres of 1/20.

Small numbers of sera, collected during the same period, in Wales and Scotland were also examined. Two of the 65 samples from Wales (3·1 %) had titres of 1/40 or greater, and 2 of 15 samples from Scotland were positive using this criterion. Thus, over the country as a whole, 43/1129 sera (3·8 %) were found to have antibody to A/Hong Kong/1/68 virus.

In swine sera collected between September 1973 and July 1977 the presence or absence of antibody was determined using the SRH test, and this part of the study employed the A/Port Chalmers/73 (H3N2) virus. The results of examinations on 4375 sera collected in every month between September 1973 and July 1977 are presented in Table 1. Overall, the percentage of positive sera recorded for 1974 was 4·5 % and for 1975 and 1976 the figures were 1·7 % and 2·3 % respectively.

When expressed in terms of the farm of origin, between 9·6 % and 4·2 % of premises yielded positive samples. Results for the first 7 months of 1977 show 9·2 % of serum samples to be positive; these occurred in 18·6 % of the premises tested.

The results of serological tests for September 1973 to July 1977 together with data reflecting influenza activity in humans (PHLS, 1973–7 unpublished) are presented in histogram form in Fig. 1. This demonstrates the seasonal fluctuation of influenza infections.

Swine sera were also tested for antibody to swine influenza viruses. Of sera examined in the HI test against A/Swine/Wisconsin/66 none had antibody. Similar results were obtained in SRH tests with A/Swine/1970/30 (HSw1N1). No serum among the 5500 tested had antibody to Swine influenza.

Table 1. *Detection of antibody to H3N2 influenza virus by the single radial haemolysis test*

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1973													
No. tested	—	—	—	—	—	—	—	—	48	69	84	59	260
No. positive	—	—	—	—	—	—	—	—	2	2	5	0	9
Percentage positive	—	—	—	—	—	—	—	—	4·2	2·9	6·0	0	3·5
No. premises	—	—	—	—	—	—	—	—	15	18	28	17	78
No. positive premises	—	—	—	—	—	—	—	—	1	2	5	0	8
Percentage positive premises	—	—	—	—	—	—	—	—	6·7	11·1	17·9	0	10·3
1974													
No. tested	111	37	78	62	57	56	67	45	84	122	133	85	937
No. positive	5	1	4	0	2	2	2	1	8	9	6	2	42
Percentage positive	4·5	2·7	5·1	0	3·5	3·6	3	2·2	9·5	7·4	4·5	2·3	4·48
No. premises	33	14	26	21	16	18	15	15	29	57	25	13	282
No. positive premises	2	1	2	0	1	1	1	1	4	8	4	2	27
Percentage positive premises	6	7·1	7·7	0	6·2	5·5	6·7	6·7	13·8	14	16	15·4	9·57
1975													
No. tested	37	59	53	156	53	104	80	30	47	50	43	52	764
No. positive	2	2	0	0	0	1	1	0	0	3	4	0	13
Percentage positive	5·3	3·4	0	0	0	0·96	1·2	0	0	6	9·3	0	1·7
No. premises	11	26	14	49	9	30	25	8	13	9	18	1	213
No. positive premises	1	1	0	0	0	1	1	0	0	2	3	0	9
Percentage positive premises	9	3·8	0	0	0	3·3	4	0	0	22·2	16·7	0	4·22
1976													
No. tested	207	209	204	90	52	76	68	47	50	70	86	80	1239
No. positive	3	2	9	7	2	1	0	1	0	0	1	2	28
Percentage positive	1·4	0·96	4·4	7·8	3·8	1·3	0	2·1	0	0	1·2	2·5	2·26
No. premises	37	37	55	29	16	12	18	14	13	12	18	10	271
No. positive premises	2	2	6	3	1	1	0	1	0	0	1	1	18
Percentage positive premises	5·4	5·4	10·9	10·3	6·2	8·3	0	7·1	0	0	5·5	10	6·64
1977													
No. tested	216	112	445	163	69	104	66	—	—	—	—	—	1175
No. positive	21	15	47	17	8	0	0	—	—	—	—	—	108
Percentage positive	9·7	13·4	10·6	10·4	11·6	0	0	—	—	—	—	—	9·19
No. premises	31	25	34	32	27	18	21	—	—	—	—	—	188
No. positive premises	10	6	9	6	4	0	0	—	—	—	—	—	35
Percentage positive premises	32·3	24·0	26·5	18·8	14·8	0	0	—	—	—	—	—	18·6

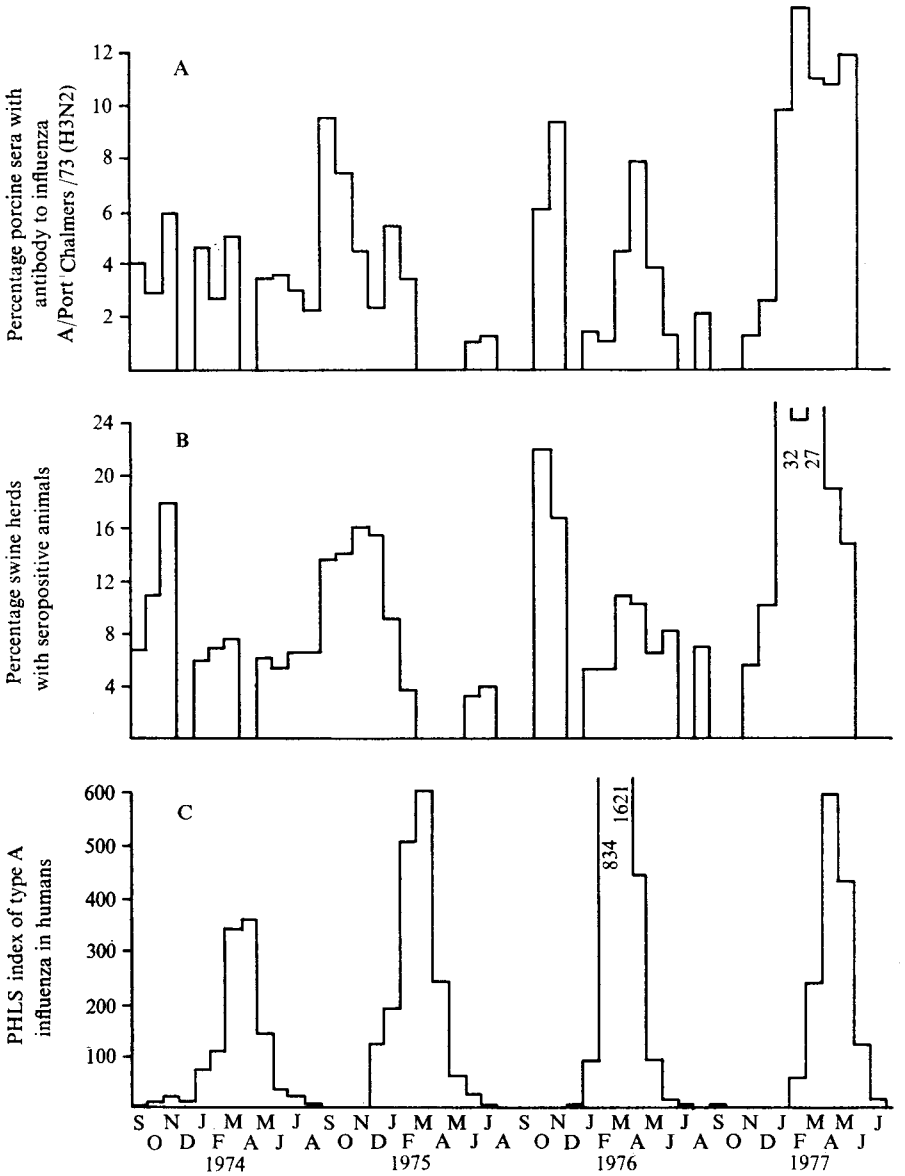

Fig. 1. (*a*) Fluctuations in the percentage of serum antibody to influenza A (H3N2) between September 1973 and July 1977. (*b*) Changes in the proportion of swine herds with seropositive animals. (*c*) Concurrent index of influenza in the human population (PHLS, unpublished).

Bovine-sera

Approximately 100 bovine sera collected in each of the years 1968–70 were examined for antibody to A/Hong Kong/68 (H3N2) virus in the HI test. Of the 294 samples, 56·5% showed inhibition at a dilution of 1/40 or greater, 43·2% inhibited at 1/80 or greater, and some 4·8% showed inhibition at dilutions of 1/640 or more. Five per cent of sera showing inhibition at dilutions of 1/40 or

https://doi.org/10.1017/S0022172400024876 Published online by Cambridge University Press

greater, and 2% of those showing inhibition at less than 1/40 were later examined in the serum neutralization test against the Hong Kong virus. No serum displayed specific neutralizing activity. The HI test results are believed to be due to the presence in bovine sera of a non-specific inhibitor not destroyed by the serum treatment employed.

In addition, 1380 bovine sera collected between November 1974 and July 1975 were examined using the SRH test. No serum produced a positive reaction against either A/Port Chalmers/73 (H3N2) or A/Swine/1970/30 (HSw/N1) viruses.

DISCUSSION

Following the infection of swine in this country with a Hong Kong-like influenza virus, the initially high percentage of porcine sera found to contain antibody in HI tests had fallen to 15% by September 1970 (Harkness *et al.* 1972). The present study shows that by March 1971 only 3·9% of porcine sera had antibody to the virus, when examined in the same fashion. Later work employed a different method of test (SRH), and also a more recently isolated variant of Hong Kong virus (A/Port Chalmers/1/73). These results show that, although the percentage of sero-positive animals remains low, there is evidence of continuing infection with H3N2 influenza viruses in swine in Great Britain.

Earlier work (Harkness *et al.* 1972) concluded that in Great Britain, as in Taiwan (Kundin, 1970), humans were the source of influenza infection for swine. The turnover in the swine population is fairly rapid; the average life of a breeding sow is of the order of 2–3 years. Consequently if interspecies transfer of virus from man is the only reason for the presence of antibody in pigs, this process would have had to have taken place repeatedly to explain the present results. Furthermore if man to swine transfer was the route of infection for pigs, the antibody prevalence in pigs might be expected to correlate with outbreaks of influenza in humans. The alternative hypothesis, which, in view of the present results must be considered more plausible, is that the virus is established in the pig population and pig to pig infections are common. A recent study (Shortridge, Webster, Butterfield & Campbell, 1977) found that Hong Kong-like viruses isolated from pigs could readily be transmitted experimentally from pig to pig. As Table 1 shows, in the years 1973–7 there are indications of a seasonal fluctuation in the prevalence among swine of antibody to influenzas with H3 haemagglutinin. The measurements are made on a different population of animals in each month, and it is difficult to be certain of the significance of these changes. Nevertheless, in the Spring of 1976, there was an increase in the prevalence of this antibody in swine at the height of a human influenza epidemic (Fig. 1). With the exception of 1977, the highest prevalence of antibody in swine occurred in each year during the autumn, up to 6 months before the peak of influenzal illness in the human population. In 1977, influenza virus activity among swine appears to have reached a maximum in February, but the human influenza epidemic was also later in the season than normal. Data concerning the influenza situation in humans is based on clinical illnesses and virus isolation figures (PHLS, 1973–7, unpublished) whereas

our data for swine is based on serological examinations; clearly the two are not directly comparable. A further complication is that pig herds in this country might justifiably be regarded as isolated sub-populations, and it is not clear whether the proportion of animals seropositive, or the proportion of herds seropositive is the better index of influenza activity, though the patterns in our results are not substantially different.

The seasonal fluctuations in influenza activity in humans and in swine could be entirely unrelated to each other and the juxtaposition of the two histograms in Fig. 1 might be of no significance. On the other hand, it may be that events in the two populations are linked in that the same factors control the development of epidemics of influenza infections in man and in swine. A third possibility is that there is a direct link, and the presence of virus in each population acts as a reservoir of infection for the other. At present there is no satisfactory way of differentiating between these hypotheses, but the situation could have sinister implications. In Hong Kong, Shortridge *et al.* (1977) have isolated from pigs variants of A/Hong Kong/1/68 virus which are no longer circulating in the human population. The pig population in Britain may similarly have become a reservoir for influenza viruses which infect humans; it may also provide a medium from which recombinant viruses could emerge. Such strains of virus could be pathogenic for swine, or for other species. The recent Fort Dix incident seems to emphasize these risks (Anon, 1976).

It seems likely that the continuing presence in swine of antibody to influenza A (H3N2) viruses is the result of both man to pig, and pig to pig transmissions, and while it is not possible to be certain which of these mechanisms is more important the evidence suggests that these viruses might persist indefinitely in pig populations. Further work will aim to establish which variants of the Hong Kong virus this porcine antibody was directed at, and to isolate the virus for further investigation.

It is clear that the swine population in Great Britain remains free from evidence of infection with swine (HSw1N1) strains of influenza A. Our results also indicate that up to 1975 cattle in Great Britain had not become involved in the epidemiology of these influenza viruses, and emphasize the advantages of the SRH test, particularly its freedom from interference from non-specific inhibitions.

We would like to thank Miss P. J. Catton for her excellent technical assistance.

REFERENCES

Anon. (1976). Current trends. Influenza. *Morbidity and Mortality Weekly Report* **27**, 55.

Chang, W. K. (1969). National influenza experience in Hong Kong, 1968. *Bulletin of the World Health Organization* **41**, 349–51.

Harkness, J. W., Schild, G. C., Lamont, P. H. & Brand, C. M. (1972). Studies on relationships between human and porcine influenza. 1. Serological evidence of infection in swine in Great Britain with an influenza A virus antigenically like human Hong Kong/68 virus. *Bulletin of the World Health Organization* **46**, 709–19.

Kundin, W. D. (1970). Hong Kong A–2 Influenza Virus infection among swine during a human epidemic in Taiwan. *Nature, London* **228**, 857.

RODEN, A. T. (1969). National experience with Hong Kong influenza in the United Kingdom, 1968–69. *Bulletin of the World Health Organization* **41**, 375–80.

SCHILD, G. C., PEREIRA, M. S. & CHAKRAVERTY, P. (1975). Single radial haemolysis – A new method for the assay of antibody to influenza haemagglutinin: Applications for diagnosis and sero-epidemiological surveillance of influenza. *Bulletin of the World Health Organization* **52**, 43–50.

SHORTRIDGE, K. F. WEBSTER, R. G., BUTTERFIELD, W. K. & CAMPBELL, C. H. (1977). Persistence of Hong Kong Influenza virus variants in pigs. *Science N.Y.* **196**, 1454–5.

WHO (1959). Expert Committee on Respiratory Virus Diseases. *World Health Organization Technical Report Series*, No. 190.