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SEROLOGICAL SURVEY IN ANIMALS FOR TYPE A INFLUENZA IN RELATION TO THE 1957 PANDEMIC

MARTIN M. KAPLAN, V.M.D., M.P.H.
A. M.-M. PAYNE, M.D., M.R.C.P.

Division of Communicable Diseases,
World Health Organization, Geneva, Switzerland

SYNOPSIS

In 1957 the World Health Organization arranged a survey of horse and swine sera in a number of countries in order to gain information on the role and importance of animals in the epidemiology of influenza. The veterinary services of the countries concerned were requested to obtain blood specimens from these animals, if possible both before and after the human pandemic of Asian influenza. This paper reports on the results of haemagglutination-inhibition and complement-fixation tests performed on these sera in WHO Influenza Centres and other collaborating laboratories.

It is apparent from these results that the Asian (A2) strain can cause natural inapparent infection in horses and swine. Equine influenza caused by the A-equi strain is also present in many countries from which it had not been previously reported, and infection in pigs with the A-swine strain, long known in the USA, has now been recorded in at least two European countries.

In a concluding section, the findings in this survey are related to other observations on the position of animals in influenza epidemiology, and future research needs are outlined.

There has been much speculation on the possible role of animals in the epidemiology of human influenza. It will be recalled that in 1918, during the second wave of the human pandemic, a large-scale epizootic of an influenza-like disease was noted in swine in the mid-west of the USA. A virus agent was established in the etiology of this disease in 1931 and was subsequently shown to be a type A influenza virus; this strain is now generally considered to represent the prototype of the influenza strain responsible for the 1918 pandemic (Shope, 1958). Since Shope's classical work three additional disease entities in animals have been shown to be caused by type A influenza virus — namely, A-equi influenza in horses (Heller

et al., 1956; Sovinová et al., 1957), classical fowl plague (fowl pest) (Schäfer, 1955), and duck influenza.[1]

When the human pandemic of 1957, caused by a distinctly new variant of type A virus, first began to spread in Asian countries during the spring of that year, the World Health Organization considered it opportune to attempt an animal serum survey in order to throw light on the animal component in the epidemiology of influenza. This decision was spurred by a report reaching WHO during June 1957 of the isolation of the variant strain from the lungs of naturally infected pigs in China, and of epizootics of an influenza-like disease in areas of China severely struck by the human disease. (Later information confirmed the isolation of the variant from swine lung tissue, but the possibility that a laboratory contaminant was involved could not be excluded. The report of influenza-like epizootics in pigs has not yet been confirmed.)

In July and August 1957, therefore, the veterinary services of a large number of countries were requested to take blood specimens from swine and horses in different parts of their countries, if possible before the pandemic struck, and to take a second specimen from the same animals, if this could be done, about three months after the epidemic had subsided in the locality. The immediate purpose of the survey was to determine whether the A2 (Asian) strain would establish itself in swine and horses—two readily available species of livestock known to be susceptible under natural conditions to other strains of type A virus (A-swine and A-equi)—and to gain some preliminary information on animal infection with these strains in different parts of the world.

Most of the veterinary services in the countries requested were able to co-operate in the survey,[2] although to a varying extent. Some were able to obtain paired serum specimens from the same animal; others could obtain only one specimen, either before or after the pandemic struck. Widely separated areas in a country were sampled in some instances, while in other countries specimens could be obtained only from a limited area. The number of serum specimens examined ranged from a few per country to hundreds, and specimens collected in some of the countries are still being held frozen for possible future examination, if this appears to be warranted on the basis of results obtained to date.

Materials and Methods

Collection and storage of serum specimens

Serum specimens were taken from animals over three months of age. The sera were separated from the blood clot as soon as possible, clearly

[1] See the article by Andrewes & Worthington on p. 435 of this issue.

[2] A list of the services collaborating in this survey is given in the Annex.

identified and recorded, and stored in the frozen state. If the latter was not possible the sera were stored at +2° to +8°C. Animals were selected predominantly near large urban centres, but animals in rural areas were also included. Random sampling in geographically or administratively well-defined areas was followed as far as practicable. Collaborators were asked to note any unusual outbreaks of influenza-like disease in swine or horses in the areas under study within recent months and to record the time in relation to the human influenza epidemic.

Pre-epidemic serum specimens and those obtained during the epidemic in the human population were taken for the most part between August and October 1957. Post-epidemic specimens were taken between three and eight months after the epidemic had subsided. The sera, in most instances held frozen, were examined in June and July 1958 in various WHO Influenza Centres and in other collaborating laboratories.

Test procedures

In order to obtain the greatest possible comparability of results, the testing laboratories were requested to follow the standard techniques recommended by the WHO Expert Committee on Influenza (1953) for the haemagglutination-inhibition (HI) and complement-fixation (CF) tests, using reagents supplied by WHO from a common stock. The following reagents were supplied:

Complement-fixation tests

(*a*) [1] Freeze-dried chorio-allantoic membrane from eggs infected with A2 virus (A/Singapore/1/57). After restoration the working dilution was 1:30.

(*b*) [1] Freeze-dried normal chorio-allantoic membrane for control purposes.

(*c*) [2] A-equi (A-equi/Praha/56) control positive serum, equine origin, giving an end-point reading (50% or less haemolysis) of 1 : 32 when tested with (*a*).

Laboratories were requested to follow the technique recommended by the WHO Expert Committee on Influenza (1953), using a 5-tube twofold dilution series, from 1:4 to 1:64, with an anti-complementary control for each serum. Different laboratories used the standard volume (1.0 ml), the small-volume test (0.5 ml) and the micro-test (0.25 ml), according to individual preference.

[1] Prepared by the Public Health Laboratory Service, Standards Laboratory, Central Public Health Laboratory, London.

[2] Supplied by the Institute of Epidemiology and Microbiology, Prague.

Haemagglutination-inhibition tests

(*d*) Haemagglutinating antigen (allantoic fluid) and control antiserum (rabbit origin) prepared with the A/Singapore/1/57 strain were supplied by a commercial company in freeze-dried form. The antigen was used for testing both horse and pig sera. Several laboratories reported that certain lots of this antigen received by them were completely inactive, and they substituted their own antigen. The potency of the antigen, as stated by the manufacturer, was 100 chicken-cell agglutinating units, and a 1 : 1000 dilution of the control serum inhibited 3 chicken-cell agglutinating units of the antigen.

(*e*) [1] HI antiserum from swine immunized with strain A/Asia/AFP/2/57 was supplied; an end-point dilution of 1: 80 of this serum inhibited the antigen described in (*d*). This serum was used as a positive control in the tests with pig sera.

(*f*) [1] HI antiserum was provided from horses immunized with A/Asia/AFP/2/57 and had an end-point titre of 1: 40 when tested with (*d*). The serum was used as a positive control for the horse serum specimens.

(*g*) [1] Haemagglutinating antigen (allantoic fluid) was prepared with A/Swine/1976/31 (Shope strain), and had a haemagglutination titre of 1 : 640 (Salk technique). This was used in the HI test for A-swine antibodies in pig sera.

(*h*) [1] HI antisera prepared by immunizing pigs with A/Swine/1976/31 had an HI titre of 1: 1280 when tested with (*g*).

Cholera filtrate, commercially prepared, was also supplied but, because it gave undependable results in some laboratories with respect to destroying non-specific inhibitors commonly found in animal sera, the use of M/90 potassium periodate was recommended, and this was found to be effective in most instances. To one volume of serum three volumes of freshly prepared unheated periodate solution were added, well mixed and allowed to stand at room temperature for at least 15 minutes. A volume of 1% glycerol-saline equal to that of the periodate solution was then added to neutralize any excess periodate. Some laboratories modified this procedure slightly (see, for instance, Italy below); usually a minimum of one-half hour was used for inactivation, an important point in the light of observations (see Poland and USA below) that 15 minutes may be insufficient to accomplish complete inactivation of inhibitor. Some laboratories preferred to destroy non-specific inhibitors by trypsinization (Jensen, 1956) although this method does not appear to be dependable in A2 infections (Jensen & Hogan, 1958). One laboratory (see USA below) reported that simple heating of horse sera to 62°C for 30 minutes was effective.

[1] Supplied by the Communicable Disease Center, Public Health Service, Department of Health, Education, and Welfare, Montgomery, Ala.

For the HI test laboratories were asked to follow the standard technique recommended by the WHO Expert Committee on Influenza (1953), using a 6-tube twofold dilution series, from 1:16 to 1:512, of at least 10 paired sera in each group of tests along with positive and negative controls. All sera were treated for non-specific inhibitor with either trypsin, cholera filtrate or potassium periodate. Minor modifications of the test procedures will be pointed out under the headings " Results " and " Conclusions from Survey Results ".

Results

General considerations

While several related incidents were reported of influenza-like disease in horses and swine occurring simultaneously with a human epidemic in a particular area, none of the animal outbreaks investigated was confirmed as being caused by A2 virus.

The accompanying table summarizes the results of tests carried out on the serum specimens collected in different countries. Before the significance of these results are analysed, some observations will be made concerning the tests performed.

As noted above, the various laboratories were requested to follow as far as possible the standard procedures for the HI and CF tests recommended by the WHO Expert Committee on Influenza (1953).[1] The HI and CF tests were selected as the most practicable procedures for the type of survey undertaken. It was hoped that the CF test would pick up any type A specific antibodies, even though it was anticipated that antibodies to the soluble antigen would reveal only fairly recent infection, and that the HI test would cover both recent and more remote strain-specific infections. Within the limitations of each type of test, to be discussed below, this seems to have been satisfactorily accomplished.

Complement-fixation test

For this test there was provided a common batch of type A antigen prepared from membranes of eggs (World Health Organization, 1953) used for the production of A2 vaccine (see page 467). (For strain-specific identification the purified antigen recently developed by Lief & Henle [2] appears to be very promising.)

Where laboratories substituted their own CF antigen the validity of the test was ascertained by the positive controls always included with each

[1] Slight modifications for these tests are described in the report of the WHO Expert Committee on Respiratory Virus Diseases (1959).

[2] See p. 411 of this issue.

SURVEY OF HORSE AND SWINE SERA FOR ANTIBODIES TO ASIAN, EQUINE AND SWINE INFLUENZA STRAINS

Test / Strain antigen / Serum specimen — Country	Horse sera *						Swine sera *			
	Haemagglutination-inhibition				Complement-fixation		Haemagglutination-inhibition			
	A2		A-equi		A2 (soluble)		A2		A-swine	
	pre-epidemic or concurrent	post-epidemic	pre-epidemic or concurrent	post-epidemic	pre-epidemic or concurrent	post-epidemic	pre-epidemic or concurrent	post-epidemic	pre-epidemic or concurrent	post-epidemic
Australia **	0/26 0/43 —	0/26 — —	— — —	— — —	0/26 1/42 —	0/26 — —	0/32 0/10 —	0/32 — 0/6	0/32 0/10 —	0/32 — 0/6
Czechoslovakia **	1/116 — —	— 0/21 0/56	101/116 — —	— 18/21 49/56	1/95 — —	— 0/12 5/51	0/60 — —	— 0/12 4/171	2/62 — —	— 0/12 1/171
Denmark **	0/7	0/7	—	—	3/80	3/80	0/156	0/156	0/156	0/156
Finland **	0/66	0/66	—	—	0/66	3/66	—	—	—	—
Germany, Federal Republic **	0/223	0/223	—	—	0/228	2/228	—	0/142	—	3/92
Greece	0/10	0/10	—	—	—	—	0/10	0/10	0/10	0/10
Ireland **	20/26	6/26	—	—	0/52	0/52	—	—	—	—
Italy **	6/25	10/25	—	—	0/9	0/9	0/27	0/27	0/27	0/27
Japan **	0/107 —	— 0/705	— —	— —	— —	— 13/717	0/99 —	— 0/737	— —	— —
Netherlands **	21/79	2/15	—	—	2/13	2/13	—	0/124	—	0/124
New Zealand **	0/34	0/34	—	—	0/34	0/34	0/33	0/33	—	—
Nigeria	0/30	0/30	—	—	—	—	—	—	—	—
Norway	0/10	0/10	—	—	0/10	0/10	0/10	0/10	0/10	0/10
Poland *	—	0/99	—	—	—	5/70	—	0/100	—	0/100
Romania **	—	0/90	—	87/90	—	21/87	—	—	—	—
Southern Rhodesia **	0/46	—	—	—	—	—	0/15	—	0/15	—
Spain **	—	—	—	—	—	—	—	0/200 †	—	—
Union of South Africa	0/6	0/6	—	—	0/6	0/6	0/9	0/9	0/9	0/9
USA **	0/228 —	0/228 —	— —	— 124/314	— —	— —	0/616 —	0/616 —	— —	137/616 —
USSR **	— Many negatives	9/9 —	— —	— —	— —	13/13 —	— —	— —	— —	— —

* The first set of figures in each column indicates the number of positive sera and the second the number of sera tested ; dashes (—) indicate that no test was done. Results of tests made on the same serum specimens are shown on the same horizontal line.

** See under " Results " in the text for analysis.

† Suspected A2 strains were isolated from the lungs of 3 pigs.

batch of sera tested, apart from the routine controls for any anti-complementary and haemolytic action of the sera under test.

The main limitation of the CF test in this survey was that the CF reaction was not as sensitive as the HI reaction (see Czechoslovakia and Romania below). Also, with the tests employed, there is a shorter-term persistence in horses of CF antibodies as compared with HI antibodies in A-equi infection (K. Raška—personal communication).

The comparability of the CF test of horse sera performed in the different laboratories can be considered acceptable, as almost all laboratories reported that the positive control serum supplied reacted at 1 : 32; a few reported end-point reactions at 1 : 16 and 1 : 64, and only one laboratory obtained a completely negative reaction with the positive control serum.

The great advantage of the CF test for survey purposes is the familiarity of unspecialized laboratories with the procedure, and the lack of complications from such factors as non-specific inhibitors and spontaneous haemagglutinins commonly encountered in the HI test. It would therefore be of great interest to pursue the possibilities opened up by Lief & Henle's CF procedure, not only from the standpoint of strain specificity but for persistence of specific antibodies in comparison with the HI test.

Haemagglutination-inhibition test

The two great pitfalls of this test lie in the choice of strains for antigen preparations and the problem of non-specific inhibitors. (An excellent review of the mechanism of the influenza haemagglutination reaction has recently been published by Buzzel & Hanig, 1958.)

Choice of strains

The most important requirement here is that the strain employed and its " phase " be reactive, i.e., inhibited by minimal amounts of specific antibody (Magnus, 1954). This fact was kept in mind when supplying the common batches of antigens to the collaborating laboratories (see above). In most of the tests recorded the positive control serum homologous to the animal serum being tested (horse or swine) was included. A second control positive serum, usually heterologous (fowl or rabbit), routinely employed in the testing laboratory, was also often included as a further check.

As previously mentioned, when testing of the animal sera was begun, several laboratories reported that the A2 antigen they received for the HI test was completely inactive, and a check revealed that one of the batches of this commercial antigen had completely lost its potency. The collaborating laboratories were advised of this and requested to substitute their own antigen in the test, and to report the end-point titres obtained with the positive control sera. Thus while end-point titres of the same positive control serum varied twofold in different laboratories (except for one

laboratory which had a fourfold variation and one which reported a completely negative reaction) the validity and comparability of the tests are considered to be satisfactory. It is of interest to note that the horse and swine positive control sera supplied for the A2 strain had a relatively low antibody content (end-points of 1 : 40 and 1 : 80 respectively), which may have been advantageous in ensuring that the tests would be sensitive enough to pick up low levels of antibody in the sera surveyed.

Non-specific inhibitors and haemagglutinins

Non-specific inhibitors and haemagglutinins in sera being tested were reported from many laboratories (see, for instance, Czechoslovakia, Japan and Poland below). The great frequency of these inhibitors in horse sera to the A2 and A-equi strain antigens employed in the survey is particularly noteworthy (see Poland and Czechoslovakia below). Treatment for these inhibitors was carried out as described under " Materials and Methods ". Laboratories varied in their preference, based on their past experience, for trypsin, cholera filtrate or periodate treatment. Most laboratories found that the periodate method was effective in eliminating the non-specific inhibitors without markedly reducing the antibody level (a twofold drop in titre was the maximum recorded). Przesmycki and co-authors,[1] however, observed that unless treatment with periodate for non-specific inhibitors in horse sera is done for at least 30 minutes the inhibitors may persist; treatment for 15 minutes only was sometimes insufficient. All the laboratories using periodate treated the sera for more than 15 minutes, except as recorded below for the USA.

Cholera filtrate treatment was effective in some laboratories and unsatisfactory in others. This variability in results can be attributed at least partially to the quality of the cholera filtrate employed (World Health Organization, 1959). One laboratory reported that trypsin-treated serum gave patterns of red cells easier to read and interpret than did the same sera treated with cholera filtrate or periodate. Several laboratories reported the presence of non-specific haemagglutinins in the sera of horses and swine to human O red cells, but these sera are not recorded in the table.

While the negative reactions recorded in the table, therefore, can be accepted with a considerable degree of confidence, the results of countries reporting positive reactions require detailed examination and analysis.

Results by country

Australia

A positive CF reaction at 1 : 4 was reported for one serum specimen from a horse. Since the remaining tests all seemed to come out uniformly negative, except for five " weak " reactions at 1 : 2, the single positive

[1] See p. 225 of this issue.

suggests the advisability of a further search for the possible presence of A-equi strain infection among equines in Australia.

Czechoslovakia

The tests recorded in the table were done very carefully with complete sets of controls for each series of tests. Non-specific agglutination of chicken erythrocytes used in the HI tests by serum alone was observed with 56 pig sera and 2 horse sera. Titres of 1 : 16 or above were considered positive in the HI test and 1 : 4 and above for the CF test (ranges encountered were 1 : 16 to >1 : 512, and 1 : 4 to 1 : 64 respectively).

The large number of positive sera in the HI tests with A-equi antigen is not surprising in view of the recorded epizootics of equine influenza caused by this strain in 1956-57 (Sovinová et al., 1957). It is noteworthy that the HI test was far more sensitive than the CF test in picking up positive reactions on the same serum specimen. The specificity of the HI test with respect to A2 and A-equi infection is equally striking. Altogether 116 sera were tested for antibodies to each strain; 101 were positive for A-equi and only one for A2. The latter reaction occurred in a specimen giving a positive reaction at 1 : 16 to both A2 and A-equi antigens. In tests made in the same laboratory, horses hyperimmunized with A-equi antigens and showing HI titres of over 1 : 8000 were never observed to have titres to A2 in comparative tests with A2 and A-equi antigens (K. Raška—personal communication). These latter results in conjunction with the specificity of the HI tests in the results reported above indicate a past infection with both A2 and A-equi strains.

Of particular interest was the finding of HI antibodies for A2 in 4 swine serum specimens (one each at titres of 1 : 32, 1 : 64, 1 : 128 and 1 : 256). These same sera were negative for A-swine. Three positive HI reactions for A-swine were observed (two at 1 : 16 and one at 1 : 64) which were negative for A2. There would appear to be little doubt of the specificity of these findings.

Denmark

Three positive CF reactions (tests were repeated) were obtained in horse sera (two at 1: 4 and one at 1: 8) using two type A antigens, one prepared in the United Kingdom and the other in Denmark. Tests were made on paired serum specimens which showed no rise or fall in titre. The three sera positive to the CF test were negative to this test using a B strain antigen; they were negative in HI tests to A antigens made with PR8, A-swine and A2 strains. Although no HI tests were carried out with A-equi antigen, it is very likely that these results indicate the presence of A-equi strain infection in equines in Denmark, as has already been observed in Sweden (Heller et al., 1956) and Czechoslovakia (Sovinová et al., 1957) and noted in the present survey in other European countries (Finland, Germany, Romania, USSR).

Finland

Three horses had a titre of 1: 4 in the CF test, reproduced in a repeated test. HI tests with A2 antigen were negative in these and all other specimens, except in two horses in which inhibition was observed in the lowest dilution in one test although none was seen in a repeated test. The positive CF titres indicate a good possibility of A-equi (or similar) strain infection.

Germany, Federal Republic

Two horse sera showed a CF titre of 1: 4, and the observation made for Finland above holds here. Three swine sera were positive to the HI test (one at a titre of 1 : 24 and two at 1 : 48) with A-swine antigen, and were negative to A2 antigen. As in Czechoslovakia (see above), this indicates the presence of A-swine type infection.

Ireland

It will be noted from the table that in horses 20 out of 26 first serum specimens and 6 out of 26 second serum specimens showed positive reactions (1: 16 or more) in the HI test. The test was performed with an A2 antigen prepared in the testing laboratory. Treatment for non-specific inhibitors was performed with potassium periodate, as indicated above. Positive reactions observed with the first serum specimens ranged from 1: 16 to 1: 512, whereas with the second specimen all six positives were recorded at 1: 16, and these occurred in horses whose first specimens had shown reactions of 1: 64 and above. The first serum specimens were collected in September 1957 and were stored until April 1958 at ordinary refrigerator temperature; in April these specimens were frozen and held at −20°C until tested in July. The second serum specimens were collected in May and frozen until tested along with the first specimens. It is difficult, therefore, to interpret the positive results, especially with respect to the first specimens, because of the possibility that even with treatment for non-specific inhibitors these substances may not have been entirely destroyed (see Poland and USA below). Further investigations would be desirable to clarify these results.

Italy

The positive HI results in horse sera with A2 antigen are of particular interest. The first serum specimens were taken in August and September 1957 and frozen; shipped to the laboratory, where they were received in a liquid state; re-frozen and held until tested in July 1958. The second specimens were taken in May and July 1958, shipped to the laboratory within a few days in the liquid state, frozen, and so held until tested with the first specimens in July. For the HI test potassium periodate treatment was used to destroy the non-specific inhibitors, as follows: 1 volume of physiological saline + 1 volume of serum + 2 volumes of KIO_4 0.5% (20 minutes at room

temperature) + 4 volumes of 10% dextrose (20 minutes at room temperature before starting to make the serum dilutions for the test). Positive reactions for the HI test were recorded starting at 1: 32 final dilution of serum. Serum controls in the test were satisfactory. The following HI titres were recorded for the positive horse specimens:

Horse serum	*First specimen* *	*Second specimen* *
Perugia 4	128	32
„ 5	64	0
„ 7	32	0
„ 8	0	32
„ 13	128	64
Teramo 2	0	128
„ 3	32	256
„ 4	64	128
„ 5	128	128
„ 6	32	128
„ 8	0	64
„ 9	0	64
„ 12	64	32
„ 13	64	128
„ 14	32	0
„ 15	32	Not done

* Reciprocal of final serum dilution inhibiting haemagglutination

These results were reproduced in a second test. It should be noted that when the first serum specimens were collected in late August 1957, the epidemic of human influenza had already started in the areas from which the horse serum specimens were obtained.

Considering all possibilities of error, including incomplete removal of non-specific inhibitors (only 20 minutes were used for periodate treatment of serum—see Poland below), the above results would appear to indicate the presence of specific inhibiting antibody for the Asian strain in at least some of the serum specimens. Further investigations would be necessary to clarify this point completely.

Japan

Thirteen out of 717 horse serum specimens collected from different parts of Japan in May and June 1958 and tested the following month showed low but definite positive reactions in the CF test. Ten of the 13 positives had reactions at 1: 4, and three at 1: 8. These results, when considered along with the large number of negative results in the HI test on swine and horse sera for A2 antibodies, indicate the probability of the presence of equine influenza caused by A-equi or a similar strain. In the HI tests, 311 swine sera and 25 horse sera showed non-specific haemagglutinins for the chicken red cells used.

Netherlands

The first serum samples from 79 horses were taken in September-October 1957, and second serum samples from 15 of these horses were obtained in January-February 1958. Twenty-one positives (sera treated with cholera-filtrate) were recorded for the HI test with A2 strain antigen, seven each at 1 : 12, 1 : 24 and 1 : 48 original dilutions of serum. Tests on 15 second specimens of these sera, which had shown positive reactions in the first specimen, revealed two positives (at 1 : 12 and 1 : 48 serum dilutions). This is hard to explain in view of the apparently long persistence of HI antibodies to A-equi infection observed in Czechoslovakia. However, shorter persistence of HI antibody may occur with a strain (such as A2) not well adapted to a particular animal species. The CF test was positive (1 : 8) in both first and second serum specimens from two horses, but only one of these animals had a positive HI reaction (1 : 24) in the first serum sample.

Mouse-protection tests were performed with first serum specimens of five horses having a positive HI reaction, as follows: strain " Mano ", Asian type, isolated in the Netherlands during the 1957 epidemic, was adapted to mice after four amniotic and four allantoic passages in hens' eggs; the 15th mouse-lung passage, having an ID_{50} of $10^{-3.8}$, was used. Serial twofold dilutions of serum were mixed with an equal value of mouse-lung suspension containing approximately 10^2 ID_{50} virus, incubated for 30 minutes at 37°C, and administered to groups of four mice by intranasal instillation. The results with these horse sera may be summarized as follows:

Horse serum	*HI test* *	*CF test* *	*Neutralization test* *
22	12	< 4	8
92	24	8	16
94	24	< 4	16
98	< 12	—	4
99	48	—	32
154	< 12	—	< 4

* Reciprocals of serum dilutions respectively inhibiting haemagglutination, fixing complement, and neutralizing approximately 100 ID_{50} of virus

There is some correlation of results between the HI test and mouse-protection test, indicating that the Asian strain was involved, but all titres are relatively low (see the tabulation below for results of experimental infection), and the possibility of the presence of non-specific neutralizing substances in the untreated sera employed in the test cannot be entirely excluded. Mouse-protection tests with these sera were repeated in an independent laboratory, and the positive results were confirmed at slightly higher titres than those recorded above. In an experimental infection of a horse inoculated intratracheally with 5 ml of undiluted allantoic fluid of an Asian strain " J.R." (isolated in the Netherlands in 1957; chicken-cell agglutination titre, 1 : 128) no detectable antibody developed within four

weeks after inoculation. However, when 10 ml of a 10% mouse-lung suspension of the " Mano " strain (see above) was similarly inoculated, antibodies developed, but no clinical illness resulted, as shown by the following figures:

Serum	*HI test* *	*CF test* *	*Neutralization test* *
Pre-infection	< 12	< 4	< 4
1 week	24	8	20
Post-infection			
2 weeks	48	16	160
3 weeks	24	8	160

* Reciprocals of serum dilutions respectively inhibiting haemagglutination, fixing complement and neutralizing approximately 100 ID_{50} of virus

New Zealand

Because of difficulties in the reading of red cell patterns in the HI reaction, repeated tests were performed. Patterns were easiest to read when trypsin treatment for non-specific inhibitors was used, although in the same test positive control sera treated with cholera filtrate or periodate gave reactions which were twofold higher or even slightly above. While the results in the table are recorded as negative in individual tests with apparently satisfactory serum controls, one post-epidemic horse serum specimen showed positive HI to A2 antigen at 1 : 32 (treated with trypsin) and 1 : 128 (treated with periodate and cholera filtrate). Four additional horse sera gave doubtful reactions in other tests. Six post-epidemic pig sera showed doubtful HI at 1 : 16 or higher with A2 antigen, as compared with pre-epidemic specimens. All these sera gave negative reactions in a final test, and are so recorded in the table.

Poland

Five positive CF reactions (all negative to HI with A2) occurred at 1 : 4 serum dilution and indicate past exposure with the A-equi strain known to be present among horses in Poland. It is noteworthy that most of the 99 horse sera tested had non-specific inhibitors (tests with untreated serum). Treatment of these sera with cholera filtrate was ineffectual in destroying these inhibitors. Periodate treatment of these sera for 15 minutes was insufficient in some cases to remove all the inhibitor, but after 30 minutes all the inhibitor was removed without affecting the antibody level of the positive control sera.[1] Cholera filtrate was apparently effective in removing non-specific inhibitor in pig sera.

Romania

The sera tested were from horses imported into Czechoslovakia from Romania two weeks prior to the taking of specimens. The results in the

[1] See the article by Przesmycki and co-authors on p. 225 of this issue.

table show the marked specificity of the HI test: none was positive out of 90 with A2 antigen, while 87 out of 90 (same sera) were positive with A-equi antigen. The HI test was positive at serum dilutions from 1 : 16 to > 1 : 512, most of them at 1 : 128 and over. The HI test was far more efficient than the CF test in picking up positive sera (87/90 and 21/87 respectively). Positive CF reactions were observed principally in those sera giving HI reactions at serum dilutions of 1 : 128 or above. These results indicate that these horses had experienced a fairly recent infection with the A-equi strain.

Spain

The collaborating laboratory reported the possible isolation of A2 virus from the lungs of three pigs. In November 1957 portions of pigs' lungs showing consolidation were collected in the Madrid abattoir and kept frozen until July 1958, when virus isolation attempts were made by amniotic inoculation ot chick embryos. Three strains of virus, isolated from the pig lung material, were inhibited by A2 positive serum only and not by sera from other A strains. These strains are being studied further. The laboratory in which the virus isolations were made had not previously been engaged in any work with the A2 strain, and the possibility of an accidental contamination was considered to be excluded. Two hundred pig sera obtained for the survey were negative in the HI test for A2.

Union of Soviet Socialist Republics

The " many negatives " noted in the table under HI tests on horse sera with A2 antigen represent serum samples taken between 1955 and 1957 from horses in Tomsk and Moscow (V. M. Zhdanov—personal communication). Elsewhere in this issue [1] will be found an account by M. G. Gaidamaka and co-authors of an outbreak of an influenza-like disease in horses in Kharkov, associated with a human epidemic in 1957 caused by the A2 virus. The information given by these authors has been incorporated in the table.

The positive results obtained with the HI test using A2 strain antigen must be discounted because of the failure to treat the sera for non-specific inhibitors. This estimate is further reinforced by a failure to demonstrate neutralizing antibodies to A2 in the positive HI sera, as stated by Gaidamaka and co-authors. As noted previously, such inhibitors are very commonly encountered in horse sera. The positive CF results indicate only that an A type of infection had been experienced, with a fairly strong possibility that the A-equi strain was involved, as this infection is known to be present in horses in the USSR. CF titres ranged from serum dilutions of 1 : 8 to 1 : 64. The non-specificity of type A antigens used for the CF test is apparent in Table 2 of the article by Gaidamaka et al. (A CF antigen made with an

[1] See p. 505.

A1 strain also gave positive reactions, whereas a B strain antigen gave negative reactions.)

United States of America

Not recorded in the table are low-level (maximum 1 : 20) HI-positive reactions with A2 antigen in 51 out of the 228 horse sera tested. None of the 228 horse serum specimens showed positive HI reactions with A-swine antigen. In many cases where these low titres with A2 antigen in the HI test were observed, there was no change between the pre- and post-epidemic specimens, or a reaction was encountered in the first specimen while the second was negative. The testing laboratory therefore considered these positive reactions to be caused by residual non-specific inhibitor despite treatment of the sera, as follows: one-half volume of trypsin (Difco 1 : 250, prepared as a 0.8 % solution in M/10 phosphate buffer at pH 8.2) was added to one volume of serum; this mixture was then held at 37° C for 30 minutes after which three volumes of M/90 potassium periodate in distilled water were added and held at room temperature for 15 minutes; this was followed by addition of three volumes of 1.0 % glycerol-saline and allowed to stand an additional 15 minutes at room temperature; 2.5 volumes of buffered saline were then added to obtain the customary initial serum dilution of 1 : 10.

Despite this treatment it is probable that some non-specific inhibitor remained, as reflected in the low titres mentioned previously. This conclusion is supported by the lack of reactions with A2 antigen in 314 horse serum specimens tested in another laboratory in the USA (see below). Also, considering the experiences in Poland mentioned earlier, treatment for 15 minutes with periodate as reported in the preceding paragraph may not have been sufficient to destroy all the non-specific inhibitor. The sera reacting at low titres were all sent from warm-weather States, and the majority of these sera were stored for up to five months in liquid form at 4°C before they were tested, which may have contributed to the persistence of these inhibitors despite treatment for them. In both laboratories the tests mentioned included adequate controls with positive sera, indicating that the A2 antigens used were satisfactory.

Pig sera showed no HI antibodies to A2, A1 (FM1), A (PR8) and B antigens, but 137 out of 166 reacted to the A-swine strain antigen. This is not surprising in view of the well-verified presence of infection with this strain in pigs in the USA. Pig sera were treated for non-specific inhibitor as described above for horse sera.

In an independent survey,[1] sera from 239 mares, 62 yearlings and 13 weanlings were tested with antigens prepared from the following virus strains: A-swine/1976/31, A-equi/Praha/56, A (PR8), A1 (FM1), A2/Formosa 313/57, A1/Denver/57, B (Lee), Sendai, Enders mumps, and

[1] This information was kindly supplied by Dr E. R. Doll, Department of Animal Pathology, University of Kentucky, Lexington, Ky., and will appear in full detail in a future publication by Dr Doll.

Dinter N virus. Except for A-equi, inhibition of agglutination at serum dilutions of 1 : 5 or greater did not occur with any virus of this group. Altogether 117 sera (49%) from mares (aged 4 to 20 years) were positive with A-equi at serum dilutions ranging from 1 : 10 to 1 : 160; 7 sera (11%) of yearlings were positive at serum dilutions of 1 : 10 to 1 : 80. Eight yearling horses inoculated intratracheally with A-equi virus did not show clinical signs of disease, but all showed HI response (1 : 40 to 1 : 80 dilutions of serum) three weeks later. Treatment of sera for non-specific inhibitors was done by heating the sera to 62° C for 30 minutes. This procedure has been employed as routine in the laboratory for several years for serological tests of horse sera. Comparative HI tests employing trypsinization, periodate treatment and heating to 56° C for 30 minutes indicated no difference as regards effect on non-specific inhibitors. The efficacy of simple heating to 62° C for the HI procedure is suggested by the negative tests obtained with other influenza virus strains as contrasted with the positive tests with A-equi.

Conclusions from Survey Results

From the preceding analysis of the results recorded in the table the following conclusions would appear to be warranted.

1. The A2 strain can apparently cause natural infection in horses. (The absence of clinical signs from both experimental and natural infections reported thus far is of interest, as contrasted with the severe clinical syndrome observed (Sovinová et al., 1957) in natural infections caused by A-equi strains.) The results analysed above for the Netherlands and Czechoslovakia, indicating the high specificity of the HI reaction for A2 and A-equi strain infections, plus confirmation of specific neutralizing antibody in the mouse-protection test in some of the Netherlands sera, would appear to justify this conclusion. The results for Italy are also strongly suggestive of natural infection, but possible incomplete removal of non-specific inhibitors cannot be excluded. The positive reactions recorded for Ireland and the USSR can be considered dubious, because of the possibility (probability in the case of the USSR) that non-specific inhibitor was involved.

2. Equine influenza caused by A-equi or a closely related A strain is present in many countries. Formerly this infection had been reported only in central and northern European countries (Sovinová et al., 1957; Heller et al., 1956). The positive HI reactions recorded in the table provide definite evidence of A-equi infection in Czechoslovakia, Romania and the USA, and the results of the CF test strongly indicate the presence of A-equi or a similar strain in Denmark, Finland, the Federal Republic of Germany, Japan, the Netherlands, Poland, the USSR and perhaps Australia.

3. The A2 strain can cause natural infection in swine (see Czechoslovakia above). Limited experiments with artificially induced infection

indicate that swine infection with the A2 strain does not cause clinical signs (Patočka et al., 1958).[1] As discussed in the following paragraph, the specificity of the positive reactions recorded would appear to be beyond doubt. As noted previously, possible isolations of A2 virus from the lungs of naturally infected pigs have been reported from Spain and the mainland of China.

4. A-swine strain infection in pigs, long known in the USA, is present in at least two European countries (Czechoslovakia and the Federal Republic of Germany). There would seem to be little doubt about this conclusion in view of the high specificity of the HI reaction in A2 and A-swine infection in pigs, as revealed in tests on the same serum specimens in all these countries (see Czechoslovakia, Federal Republic of Germany and USA in the table). Also, the large number of HI-negative results in swine sera for A2 and A-swine in these and other countries testifies to the adequacy of treatment for any non-specific inhibitor which may have been present.

Epidemiological Considerations

There has been no lack of speculative efforts concerning the origins and inter-epidemic reservoirs of virus strains causing epidemics, and we shall not attempt here to review all the various theories. It is clear that any valid theory will have to take into account the animal influenzas as part of the natural history of the disease. It is equally clear that relatively little is known of naturally occurring influenza in animals, despite the outstanding series of observations by Shope (1958) on swine influenza and by Czechoslovak workers on equine influenza (Sovinová et al., 1957; Sovinová & Ludvík, 1958).[2]

The survey discussed in this paper was undertaken for the purpose of gathering information on two animal species, swine and horses, known to be involved in influenza epidemiology, and the pandemic of 1957 provided an excellent opportunity to add to our knowledge of this subject. Despite the very limited number of animals surveyed in various countries valuable data have been gathered and many leads have been revealed for further research. In the following discussion we shall attempt in summary form to combine these findings with certain other observations relating to the position of animals in influenza epidemiology, with a view to focusing attention on such leads for future research.

The distinct difference of the A2 strain from all human A strains previously isolated, particularly its non-sensitivity to the beta-inhibitor found in normal animal serum, and its reported isolation from the lungs of naturally infected swine in China early in the pandemic, provided compelling reasons

[1] See also the article by Wallace & Kissling on p. 455 of this issue.

[2] See also the article by Tůmová & Fišerová-Sovinová on p. 445 of this issue.

for the survey described in this paper by suggesting the possibility that the A2 strain originated in an animal reservoir. Mulder and his colleagues in several recent publications have given particular emphasis to this possibility, and point out several linked observations in support of this view. These include (*a*) serological studies in aged people, indicating that closely related viruses were responsible for the 1889 and 1957 pandemics (Davoli & Corsi, 1957; Davenport & Hennessy, 1958; Mulder & Masurel, 1958); (*b*) the common country of origin (China) of these two pandemics, and presumably those of 1830 and 1782 (Mulder & Masurel, 1958); (*c*) the non-sensitivity to the beta-inhibitor found in normal human and animal sera of straight egg-lines of A2 and A-swine (1918 pandemic prototype) viruses, and the potential pneumotropism and virulence of the strains as distinct from other human A strains studied (Hers et al., 1958; Mulder et al., 1958) and (*d*) the antigenic stability of A-swine strains isolated from pigs in the USA since 1930 (Jensen & Petersen, 1957). (Also of interest are serological studies (Nelson & Lewis, 1958) which indicate the closer relation of A2 to A-swine infections than of A2 to other A types predominant since 1935.) Taking into consideration the current theory that step-by-step variation in human A strains causing epidemics in recent years is influenced by antibodies in human population groups produced by dominant antigens of previous strains, Mulder & Masurel (1958) postulate that the great pandemics may have arisen out of a particular A strain existing in Asia, presumably in an animal carrier.

The preceding considerations point to the possibility that a parent influenza strain, which would no doubt be of great potential significance for immunization purposes, resides in an animal reservoir on the mainland of China. The best approach towards investigating this possibility would lie in an intensive study of the wild and domestic fauna in that area by means of serological, ecological and epizootiological studies combined with attempts at virus isolations. It is to be hoped that such a study will be undertaken at the earliest opportunity so that the answer might not be lost through the possible dying off of a particular generation of animal species which may have been involved. The likelihood of this would not appear to be great if an epizootiological situation exists analogous to that of swine influenza in the USA, where the A-swine strain has shown remarkable stability.

As pointed out elsewhere in this issue by Tůmová & Fišerová-Sovinová,[1] there is apparently a haemagglutinating component, in addition to the common soluble antigen as revealed in the CF test, shared by the strains A2 and A-equi, and by those of duck influenza and fowl plague. Tůmová and colleagues observed that only A2 and A-equi, out of several different A strains (including A-swine), agglutinated calf, horse and pig red cells.

[1] See p. 445.

The reaction with horse cells was particularly striking as a differential characteristic, and they observed this to be true of two other animal influenza A viruses—those of duck influenza and fowl plague. Wang & Lin (1958) noted that A2 and not A or A1 human strains agglutinated sheep, goat, horse, cow, pig, rabbit and cat cells. Their A2 strains varied in sensitivity to non-specific inhibitors found in sera of different animal species, although J. Mulder (personal communication, 1959) observed that A2, A-equi, A-swine and duck influenza strains were all insensitive to beta-inhibitor of normal ox serum which affected all other human A strains tested.

Apart from the further comparative antigenic analysis of animal influenza strains that is obviously indicated, the epizootiology of A-equi infection in horses should be thoroughly investigated, particularly from the standpoint of world prevalence and natural and experimental infection in horses and other animals. Such studies should be applied equally to fowl plague (Schäfer, 1955) and duck influenza,[1] which have been shown to be caused by type A influenza virus.

It is generally believed that the A-swine virus represents the prototype of the virus which caused the 1918 human pandemic, and that the disease in swine originated from human infection (Shope, 1958). This view is based principally on the circumstantial evidence that the classical picture of swine influenza now observed in natural infections was never noted elsewhere prior to its appearance in the USA during the 1918 pandemic, and on the seeming absence of swine influenza since that time outside the USA. Such a view presupposes the occurrence of a remarkable set of circumstances—all the more remarkable if the hypothesis is extended to include the limitation to a single country (USA) of the introduction of infection from man to swine and also to include the unusual cycle observed in natural swine infection and described by Shope (occult virus passed through lungworm larvae and the earthworm to the lungs of pigs). The reverse possibility—of infection from swine (or other animals) to man—should therefore be kept in mind. Actually very few attempts have been made to search for or to study A-swine infection in pigs outside the USA. The present survey indicates that A-swine infection is found naturally in pigs in at least two European countries (Germany and Czechoslovakia), but the possibility exists of introduction into these countries of infection from pigs or pork products originating in the USA. The striking gaps in knowledge as to possible natural infection in pigs in other parts of the world, especially in areas where introduction of the disease from the USA could be excluded, call for further surveys for this disease and, should it be found in such areas, for detailed investigations to determine the natural transmission cycle.

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[1] See the article by Andrewes & Worthington on p. 435 of this issue.

Shope and others have shown previously that swine are susceptible to natural infection with influenza virus A causing epidemics. As has been indicated above, horses and swine are both naturally and artificially susceptible to infection with the A2 strain. Further studies are therefore indicated to determine whether A2 infection has indeed established itself as a continuing disease in horses and swine, as well as perhaps in other animals. Serological screening of serum specimens from wild and domestic animals from different parts of the world to detect influenza antibodies should be the basis for such studies.

The knowledge that four distinct disease entities occur naturally in animal populations (swine influenza, equine influenza, duck influenza and fowl plague) calls for a re-examination of all likely virus infections in animals to determine any possible relationships with influenza strains.

For the clarification of the natural history of influenza, so urgently needed, this problem of the animal influenzas can no longer be neglected. Investigations along the lines indicated above will certainly add much to our knowledge of influenza epidemiology, and the World Health Organization hopes to stimulate and co-ordinate such studies in the future.

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Annex

SERVICES PARTICIPATING IN SEROLOGICAL SURVEY

ARGENTINA	Department of Veterinary Services, Ministry of Agriculture.
	Pan-American Zoonoses Centre, Azul.
	Ministry of Health.
AUSTRALIA	*Commonwealth Serum Laboratories, Victoria.
BELGIAN CONGO	Service Vétérinaire, Laboratoire d'Elisabethville.
BRAZIL	Department of Veterinary Services, Rio de Janeiro.
	Ministry of Health.
CANADA	Department of Agriculture, Health of Animals Division, Ottawa.

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CHILE	Department of Veterinary Services, Ministry of Agriculture.
	Veterinary Public Health Services, Ministry of Health.
CZECHOSLOVAKIA	Institute of Epidemiology and Microbiology, Prague.
	State Scientific Veterinary Institute, Bratislava.
DENMARK	* Statens Seruminstitut, Copenhagen.
	Veterinaerdirektoratet, Copenhagen.
FINLAND	* State Serum Laboratory, Helsinki.
	Veterinary Services, Ministry of Agriculture, Helsinki.
FRANCE	* Institut Pasteur, Paris.
	Service Vétérinaire, Ministère de l'Agriculture.
GERMANY, FEDERAL REPUBLIC	* Robert Koch Institute of Hygiene and Infectious Diseases, Berlin.
	Veterinary Medicine Department, Max von Pettenkofer Institute, Berlin.
GREECE	* Central Public Health Laboratory, Ministry of Hygiene, Athens.
	Veterinary Services, Ministry of Agriculture, Athens.
ICELAND	Institute for Experimental Pathology, University of Iceland, Reykjavik.
INDIA	Indian Veterinary Research Institute, Mukteswar.
	Pasteur Institute of Southern India, Coonoor.
IRELAND	Veterinary Research Laboratory, Dublin.
	* Virus Research Laboratories, University College, Dublin.
ITALY	Servizi Veterinari, Alto Commissariato per l'Igiene e la Sanità Pubblica, Rome.
	Università di Firenze, Istituto di Microbiologia, Florence.
JAPAN	Animal Hygiene Section, Bureau of Animal Industry, Ministry of Agriculture and Forestry, Tokyo.
	* Japanese Influenza Centre, National Institute of Health, Tokyo.
MALAYA	Institute for Medical Research, Kuala Lumpur.
	Veterinary Services, Kuala Lumpur.
NETHERLANDS	* Netherlands Institute for Preventive Medicine, Leiden.
	Veterinary Services, Ministry of Agriculture, Food and Fisheries, The Hague.
NEW ZEALAND	Department of Microbiology, University of Otago Medical School, Dunedin.
	* National Health Institute, Wellington.

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NIGERIA	Veterinary Services, Department of Agriculture.
	West African Council for Medical Research, Yaba, Lagos.
NORWAY	State Institute of Public Health, Oslo.
PARAGUAY	Department of Veterinary Services, Ministry of Agriculture.
	Ministry of Health.
PERU	Department of Veterinary Services, Ministry of Agriculture.
	Ministry of Health.
POLAND	Institute of Hygiene, Warsaw.
	State Institute of Rural Occupational Medicine and Rural Hygiene, Lublin.
	Veterinary Services, Ministry of Agriculture, Warsaw.
SOUTHERN RHODESIA	Veterinary Services, Salisbury.
SPAIN	* Escuela Nacional de Sanidad, Facultad de Medicina, Madrid.
SWEDEN	Statens Veterinärmedicinska Anstalt, Stockholm.
UNION OF SOUTH AFRICA	Onderstepoort Veterinary Laboratory, Pretoria.
	* South African Institute for Medical Research, Johannesburg.
UNION OF SOVIET SOCIALIST REPUBLICS	* Influenza Centre for the USSR, Institute of Virology, USSR Academy of Medical Sciences, Moscow.
UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND	Central Veterinary Laboratory, Ministry of Agriculture and Fisheries, Weybridge, Surrey.
	Public Health Laboratory Service, Colindale, London.
	* World Influenza Centre, National Institute for Medical Research, London.
UNITED STATES OF AMERICA	Department of Animal Pathology, University of Kentucky, Lexington, Ky.
	* Communicable Disease Center, Public Health Service, US Department of Health, Education, and Welfare, Montgomery, Ala. (International Influenza Center for the Americas), and Atlanta, Ga.
URUGUAY	Department of Veterinary Services, Ministry of Agriculture.
	Ministry of Health.

* WHO Influenza Centres.

RÉSUMÉ

Les auteurs rendent compte du résultat d'enquêtes effectuées dans divers pays, à la demande de l'OMS, pour la recherche des anticorps et des virus grippaux chez les animaux domestiques au cours de la pandémie 1957/58. Le rôle des animaux comme réservoir

du virus grippal est soupçonné depuis longtemps. En 1931, on a montré qu'une épizootie des porcs, aux Etats-Unis, était due à un virus grippal de type A. On a même considéré ce virus comme le prototype de celui qui a causé la pandémie de 1918. Depuis lors, trois maladies animales ont été reconnues comme dues au virus A: la grippe du cheval (A-equi), la grippe du canard et la peste des poules. Lorsque la pandémie éclata en 1957, l'OMS intervint auprès des services vétérinaires d'un grand nombre de pays, leur demandant de faire prélever, avant l'apparition de l'épidémie sur leur territoire, si possible, puis après l'épidémie, des sérums de porcs et de chevaux, afin d'y rechercher les anticorps grippaux, indices d'une multiplication du virus A2 chez ces animaux, connus comme naturellement sensibles au virus grippal.

Les auteurs indiquent la technique suivie pour prélever, conserver les sérums et pratiquer les tests d'inhibition de l'hémagglutination. Après avoir exposé les résultats obtenus dans divers pays les auteurs concluent que le virus A2 peut infecter naturellement le cheval. La grippe du cheval — causée par A-equi ou un virus A étroitement apparenté, existe dans plusieurs pays. Le virus A2 infecte naturellement le porc. L'infection expérimentale, cependant, n'a pas provoqué de manifestations cliniques. La grippe A du porc, connue depuis longtemps aux Etats-Unis, existe aussi en Tchécoslovaquie et en Allemagne.

On connaît encore très peu la grippe chez les animaux. La différence très nette entre les virus A2 de la récente pandémie et ceux des épidémies précédentes, et le fait qu'il a été trouvé chez le porc, en Chine, tout au début de l'épidémie, suggèrent que le virus A2 s'est répandu à partir d'un réservoir animal. Divers faits appuyent cette manière de voir: les études des sérums de personnes âgées, montrant une étroite parenté entre les épidémies de 1889 et de 1957; l'origine commune (la Chine) de ces deux pandémies et probablement de celles de 1830 et 1782, l'insensibilité à l'inhibiteur bêta du sérum normal d'homme et d'animal, de souches A2 et A-porc (prototype 1918) cultivées en série sur œuf; la stabilité antigénique des souches A isolées sur les porcs depuis 1930 aux Etats-Unis. Pour d'autres raisons encore, on suppose que la pandémie a pris naissance à partir d'une souche existant en Asie, probablement dans un réservoir animal. On pourrait donc concevoir qu'une souche-mère de virus grippal existe en Chine, dans un animal réservoir — ce qui ouvrirait des possibilités immenses à l'immunisation si cela se vérifiait. Il y aurait lieu d'attaquer ce problème en étudiant la faune domestique du point de vue sérologique, écologique et épizootique, et en tentant d'isoler les virus. Les auteurs suggèrent plusieurs sujets précis de recherches dans ce domaine. On ne peut plus ignorer le rôle des animaux dans l'épidémiologie de la grippe et il est urgent que des études approfondies viennent le préciser.

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