Organisation of worldwide surveillance of equine influenza*

The meeting was mainly devoted to equine influenza, in line with the working programme of the Group of Specialists in Horse Diseases, approved by the International Committee of the O.I.E. at its 49th General Session.

I. LIST OF PARTICIPANTS
(See page 445)

II. EXPERTS’ REVIEWS AND DISCUSSIONS
A. AETIOLOGY OF EQUINE INFLUENZA
(Dr B. Tumova)

Equine influenza is a contagious viral disease affecting the respiratory tract of horses. It mainly occurs in areas where vast numbers of horses are found.

The two causal viruses recognised today belong to the orthomyxovirus group and are known as Equi 1 and Equi 2. Both of these agents have identical morphology, similar epizootiology and pathology. They however differ greatly in their isolation and their role in epidemics; they also possess distinct envelope antigens.

Virus Equi 1 (A/Equi/Prague/56) was isolated in Czechoslovakia in 1956 and spread to many regions of the world between 1956 and 1977.

Virus Equi 2 was first isolated in the USA in 1963 and gradually spread to practically all continents. Australia appears to be the only free continent.

The dissemination of both equine influenza subtypes can thus be considered to be almost worldwide. However, there have been relatively few reports of large outbreaks in recent years, probably as a result of vaccination. Local outbreaks occur and spread from one herd to another (depending on variations in immunity) especially during the horse-racing season. Transport and training stress contribute to outbreaks of disease.

As very little is known about virus persistence and about inapparent forms of the disease, the efficiency of veterinary control and quarantine measures is entirely relative.

The two subtypes may be found in a single outbreak. This phenomenon, demonstrated for the first time in equine influenza, was later revealed in influenza of birds and pigs and recently in man. One should bear in mind that recombinants may be at the origin of this co-existence of the two subtypes.

Furthermore, very little is known of the origin of equine influenza viruses. A retrospective serological survey proves that Equi 1 had been present long before it was isolated. On the contrary, Equi 2 having left no serological trace before it was isolated in 1963 appears to be quite new.

Both equine viruses have the characteristic morphology and structure of the orthomyxoviruses; their outer membrane carries the characteristic antigens: haemagglutinin and neuraminidase which play an important part in the pathogenesis of infection, as well as in the development and nature of immunity. Changes in these antigens take place independently and are co-responsible for failures in immunity and vaccination. The genome is an RNA consisting of 8-9 distinct segments which encode 7 structural and 1 or 2 non-structural proteins. Genome characterisation is at present the leading method for tracing the antigenic profile of different strains and for pursuing epidemiological surveys. Characterisation has not as yet been sufficiently applied to equine influenza.

Current knowledge of the physicochemical and biological properties of the equine viruses is incomplete. Like the other influenza strains, both equine strains are sensitive to ether and acids but they are more stable at lower pH; the haemagglutinin of Equi 1 is particularly stable.

Equine strains can agglutinate the erythrocytes of many different animals. Both readily infect the usual laboratory animals, pigs and some domestic and wild birds. Man seems to be susceptible to experimental infection by Equi 2; however natural infection has not been reported. On the contrary, zebras, donkeys and camels may be infected naturally (as found in zoos). Serological evidence of infection of camels was reported in Algeria.

The two equine virus subtypes differ in their surface antigens. This means that a difference in RNA segments 4 and 6, encoding these antigens, is involved: this also provides evidence of a different origin of the two viruses. By its haemagglutinin Equi 1 is related to the subtype Hav 1 group of avian influenza viruses. Equi 2 has been shown to possess 80% base-sequence homology with human H3 and avian Hav 7 viruses.

In the new nomenclature, these three subtypes are classified into one subtype H3. The neuraminidase, particularly of Equi 2, has shown similarities to that of some avian influenza isolates. An avian origin of Equi 2 through recombination is a highly probable hypothesis.
Antigenic variation (« drift ») does exist, despite the fact that it is less frequent than in human viruses. Drift was demonstrated in Equi 2 strains in South America in 1969 and in Japan in 1971. The more stable Equi 1 did however show changes in England in 1972 and in Czechoslovakia in 1978. The detection of antigenic drift has practical implications for the quality of serological diagnosis and especially for the production of effective vaccines.

Equine influenza prevention therefore implies the constant surveillance of equine influenza outbreaks throughout the world, the determination of antigenic properties and of possible drift as well as the prompt exchange of information and strains. This is all the more important as the emergence of new subtypes or recombinants cannot be precluded.

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During the discussion that followed Dr. Tumova’s review, it was confirmed that antigenic drift does occur in equine strains, as with human strains (numerous examples were given by participants). Professor Hannoun emphasised the importance of promptly applying surveillance methods to equine viruses, similar to those used by the W.H.O. for human viruses. He also stated the need to adopt techniques used for human influenza, i.e. surveys of RNA base-sequences and the use of monoclonal antibodies for example. In these studies it must be remembered that successive passages in eggs may modify the virus.

Each isolated strain should be identified and compared with others. For vaccination purposes, the best strain is not necessarily the most recently isolated but rather that which shows the highest immunity response by way of its antigenicity. Variability differences between human and equine strains is explained by the fact that immunological pressure is much lower in horses.

B. ECOLOGY OF EQUINE INFLUENZA  
(Pr. C.L. Hannoun)

The new classification of influenza viruses adopted by the W.H.O. is based on unifying the nomenclature of haemagglutinins and neuraminidases while recognising the close relationship between influenza strains of different species. For example the Equi 2 haemagglutinin is very similar to the H3. The concept of influenza viruses being linked to species is replaced by the more unified conception of a single group of influenza viruses including strains of differing virulence depending on the animal species involved.

The new nomenclature of haemagglutinins and neuraminidases is summarised in Tables I and II.
TABLE I

Nomenclature of haemagglutinins

<table>
<thead>
<tr>
<th>Subtypes in the present system</th>
<th>Subtypes in 1971 system</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>Ho, H1, Hsw1</td>
</tr>
<tr>
<td>H2</td>
<td>H2</td>
</tr>
<tr>
<td>H3</td>
<td>H3, Heq2, Hav7</td>
</tr>
<tr>
<td>H4</td>
<td>Hav4</td>
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<tr>
<td>H5</td>
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<td>H6</td>
<td>Hav6</td>
</tr>
<tr>
<td>H7</td>
<td>Heq1, Hav1</td>
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<tr>
<td>H8</td>
<td>Hav8</td>
</tr>
<tr>
<td>H9</td>
<td>Hav9</td>
</tr>
<tr>
<td>H10</td>
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</tr>
<tr>
<td>H11</td>
<td>Hav3</td>
</tr>
<tr>
<td>H12</td>
<td>Hav10</td>
</tr>
</tbody>
</table>

TABLE II

Nomenclature of neuraminidases

<table>
<thead>
<tr>
<th>Subtypes in the present system</th>
<th>Subtypes in 1971 system</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>N1</td>
</tr>
<tr>
<td>N2</td>
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</tr>
<tr>
<td>N3</td>
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<tr>
<td>N5</td>
<td>Nav5</td>
</tr>
<tr>
<td>N6</td>
<td>Nav1</td>
</tr>
<tr>
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<td>Neq1</td>
</tr>
<tr>
<td>N8</td>
<td>Neq2</td>
</tr>
<tr>
<td>N9</td>
<td>Nav6</td>
</tr>
</tbody>
</table>

The two equine viruses therefore become:

Heq1 Neq1 = H7 N7
Heq2 Neq2 = H3 N8

Interspecies virus transmission can occur: it has been proved between man and pigs and probably exists in many other cases. Strains possessing « equine » haemagglutinins or neuraminidases have been isolated in birds.

Interchanges between influenza viruses are also possible. Genetical reassortments are facilitated by the segmented nature of the genome and by the fact that double infections have been reported in man and in many animal species, notably horses. For example in man, a strain which had been classified between the Texas and Bangkok strains was, in reality, a combination of
two strains. Only at cloning was it shown to be a mixture of a strain similar to the Texas strain and of a « true » Bangkok virus. In a person immunised against « Texas » and infected with such a mixture, the result of a Bangkok infection could be interpreted as a mutation whilst it is more likely a selection.

In support of the discussion on antigenic drift, Professor Hannoun commented the results of the H.I. test where five equine virus strains were used with corresponding sera (Table III).

**Table III**

*Antigenic drift of the equine influenza virus*

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>SERA</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Miami</td>
<td>2350</td>
<td>2520</td>
<td>479</td>
<td>390</td>
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<tr>
<td>New York</td>
<td>513</td>
<td>1910</td>
<td>224</td>
<td>224</td>
<td>390</td>
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<tr>
<td>Alfort</td>
<td>317</td>
<td>1550</td>
<td>892</td>
<td>447</td>
<td>417</td>
</tr>
<tr>
<td>Tokyo</td>
<td>630</td>
<td>6170</td>
<td>892</td>
<td>3100</td>
<td>2520</td>
</tr>
<tr>
<td>Algiers</td>
<td>296</td>
<td>2050</td>
<td>630</td>
<td>590</td>
<td>1780</td>
</tr>
</tbody>
</table>

Table III reveals the most interesting strains for vaccination purposes (New York strain).

**In the discussion that followed, questions were raised on how influenza commences in a herd and on the role of vectors and particularly of birds. Between 1964-1965 the origin of equine influenza outbreaks was explained by the movement of horses, which brought Equi 2 to Europe.**

With regard to human influenza, systematic sampling has shown the persistence of the virus in periods of disease absence. For example, a virus strain may be isolated two months before the disease breaks out. The fact that the virus can be carried in this way is very important as reactivation of the virus may occur by recombination of persistent strains. Experimentally, reactivation was demonstrated from a virus inactivated by ultra-violet and placed in the presence of a human virus.

According to Professor Bryans, an outbreak occurred in experimental horses which had been isolated for six months; this suggests that the virus may have been reactivated by a human virus.
Professor Bachmann confirmed that influenza viruses in swine may remain present in the pig without any clinical signs. Thus virus persistence can occur during the summer season without implying the possible participation of earth worms (Shope).

On this topic, Professor Hannoun described experiments showing the persistence and excretion of the human virus in pigs:

a) pigs may be infected with a human virus (A/Texas/H3 N2) without showing clinical signs of disease;

b) infection through contact with affected animals can take place immediately and the virus may very soon be found in the throat and then excreted;

c) the carrier state, which is almost asymptomatic, can be prolonged for at least 25 days with titres enabling isolation in eggs;

d) the presence of serum antibodies, even at very low levels, may considerably change the onset and development of viraemia, prolong the period of excretion from respiratory tracts and inhibit the formation of antibodies with higher titres.

The persistence of human viruses in pigs was confirmed by Professor Bachmann. This has not been reported in horses, but has not been examined to date. A study on horses as possible carriers would be of interest.

C. DIAGNOSIS OF EQUINE INFLUENZA
(Pr. P.A. Bachmann)

Diagnosis of equine influenza is based on virological and serological methods. However, since two different equine influenza subtypes exist and antigenic drift is reported to occur, more advanced differentiation techniques are required.

Virus isolation does not pose too many problems when performed during an outbreak, using nasal swabs which are taken at the beginning of the acute phase of the disease. Swabs are thoroughly soaked in transport medium and supernatant is inoculated into the amniotic or allantoic cavity of 9-11 day-old chicken embryos. After incubation at 35°C for 3 days, the amnioallantoic fluid is collected and checked for HA activity using chicken red blood cells. Two to three successive passages in eggs can be made.

The procedures to be followed and sampling techniques during non-epidemic periods or in cases of inapparent disease have not yet been established. This point should be emphasised in the recommendations.

For the demonstration of antibodies, haemagglutination-inhibition tests (HI) are performed using Equi 1 and Equi 2 antigens. Development of antibodies is judged by analysing two sera collected at 2-3 week intervals. Four-fold antibody increase between the first and second serum sample is significant.
The use of ether-treated antigens gives the best results.

In an epidemiological survey, the differentiation of virus isolates can be undertaken by determining the nucleoprotein (NP) antigens; this is most conveniently done by immunodiffusion. Most important however is the differentiation of the haemagglutinin (HA) and the neuraminidase (NA). Immune sera from ferrets are the most suitable for revealing antigenic drift within a subtype, but they are not easily obtainable. Rabbit sera may however show clear differentiation between strains.

The neuraminidase-inhibition test is advised for studying recombinant strains.

For a more precise differentiation of strains, other methods should be used, notably:
1. studying RNA composition;
2. using monoclonal antibodies (this method is used for influenza strains of pigs and gives excellent results).

In conclusion, Professor Bachmann suggested that:

a) identification and classification of virus isolates be undertaken at a central reference laboratory;

b) reagents be prepared against all virus isolates differing from reference strains A/Equi/Prague and A/Equi/Miami 63;

c) a classification of these viruses be pursued;

d) standard reagents (antigens and antibodies) be distributed to different laboratories for diagnostic purposes;

e) a pattern of monoclonal antibodies be produced to enable detailed isolate differentiation and classification.

Discussion followed on various techniques which could be used to improve equine influenza diagnosis:

(i) detection of IgM which show infection would be interesting but reagents (anti-IgM serum) for horses are either inexistent or of poor quality;

(ii) the radial haemolysis technique may be envisaged for equine influenza diagnosis. At present, results obtained are seldom reproducible;

(iii) immunofluorescence would be a suitable method enabling identification of infection directly from nasal mucous (as in pigs) but a conjugate against the Ig of horses is required;

(iv) the ELISA test could also be studied.
D. EQUINE INFLUENZA VACCINATION
(Dr. M. Bonneau)

At present the vaccine is produced as a mixed vaccine (A/Equi 1 and A/Equi 2); the culture is obtained in embryonated eggs. After inactivation of the mixture of antigens, the adjuvant is added.

Control of its activity on horses poses the problem of finding animals which are certified to be free from disease.

In the future, the use of recombinants and even hypothetical recombinants A1 A2 is envisaged, which would cumulate the antigenicity of the two subtypes instead of present strains.

With regard to production, cell replication (and no longer replication on embryonated eggs), may be envisaged to obtain purification (after inactivation) through chromatography, thus achieving a sub-fraction of the virus (haemagglutinin-neuraminidase) which should then be concentrated.

In the fairly near future, and on account of recent findings on the E.L.A. system of horses, attention should also be paid to the possible relationship between these leucocyte antigens and immunological response.

In Professor Bachmann's view, testing vaccin potency remains a controversial topic: systematic control in horses comes up against the problem of finding horses with no antibodies. Furthermore, subsequent controls of vaccinated or non-vaccinated herds are unsatisfactory. In vitro tests must be performed to demonstrate vaccine potency.

Is production on cell cultures useful and worthwhile? Attempts to adapt virus strains to cells have resulted in the production of incomplete viruses and in insufficient amounts. This implies a higher concentration of viruses and consequently increased cost prices.

It is obvious that in ovo production necessitates purification and the elimination of egg proteins. Dr. Tumova considered that studies on more advanced purification methods and production in embryonated eggs would be more worthwhile, rather than considering production in cells.

Dr. Bonneau however insisted on the advantages of cell cultures from an industrial point of view, and even more so as the problem of obtaining an incomplete virus is not that important if useful antigens are produced. The possibility of large-scale production should adequately compensate the slightest yield of cell cultures in comparison with egg cultures.

The use of recombinants for vaccine production is possible; this has already been proposed but manufacturers found it unsuccessful, apparently because of the major technological changes it implies for them and because of the increased cost price of vaccines. However, Professor Bachmann considered that ten times as many antigens could be obtained by using recombinants compared to the amount obtained using common strains. A recommendation could be formulated on the use of recombinants.
Nonetheless, strains used should be constantly re-assessed in connection with the epidemiological situation.

Protection is dependent on the quality of the vaccine, on vaccination schedules (which are not always respected) and on intercurrent diseases (herpes and allergic coughs) which may complicate an assessment of protection levels.

Professor Bryans considered that in the present circumstances vaccine potency can only be evaluated through HI antibody response. The question however remains to determine the minimum titre required; all the more so as protection is not necessarily in proportion to the HI titre.

Dr. Tumova stated that doses of antigen in the vaccine should be expressed in µg; for example, giving the level of haemagglutinins, but as this quantity of haemagglutinins may only be determined by a quantitative immunological reaction, as in radial haemolysis, sera purification should also be considered. Research work should be undertaken on the quantity of antigens required. The precise maximum quantity of egg protein should also be stated.

Testing of the purity and potency of the vaccine should be undertaken by a laboratory which is independent from the manufacturers whilst however maintaining liaison with them, to use the same methods if nothing else. To date, vaccine standardisation and procedures have not been satisfactorily dealt with.

Dr. Bonneau also stated that an effort should be made to evaluate cellular immunity, either in vivo or in vitro. It would be interesting for manufacturers to test this cellular immunity in vitro. For present practical purposes, participants considered that the problem of humoral immunity should be now resolved.

Whilst recognising that vaccination schedules largely depend on the quality, standardisation and potency testing of vaccines, the following problems should be kept in mind:

1. from what age may vaccination commence?
2. vaccination of brood-mares;
3. vaccination of sports horses.

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The following conclusions were made with regard to vaccination:

(i) adapt the choice of vaccinal strains to the epidemiological situation;
(ii) define the minimum norms for antigen in the vaccine, as in human influenza;
(iii) create a working group for vaccine testing;
(iv) define a vaccination schedule in line with results obtained.

III. RECOMMENDATIONS ADOPTED BY THE EXPERTS

Equine influenza is recognised throughout the world as being a serious economic hazard for horse racing authorities and in general for all those with race-horse interests. Although equine influenza vaccines have been available in most countries for fourteen years, the efficacy of various vaccine preparations is recognised to vary greatly. The efficacy of vaccines is also compromised by antigenic changes in both types of equine influenza viruses. It is quite clear that the presence of a new antigenic type would cause worldwide equine influenza spread, as happened in the sixties. Equine influenza control is henceforth conditional upon the establishment of an efficient international surveillance system and upon the exchange of information and research designed to obtain standardisation of vaccines of maximum potency and safety. Furthermore, if efficient equine influenza control is to be successful, competent scientists must be recruited and be assisted in their research programme by organisations concerned about the economic problems this disease implies.

The experts recommend that:

1. Diagnosis be standardised by the definition of test procedures and the availability of reference antigens and antibodies.

2. The need for a surveillance system based on virus isolation, identification of subtypes and variants be emphasised.

3. A classification scheme and final differentiation schedule for virus isolates be worked out in order to advise producers to use strains of current epidemiological status for vaccine production.

4. An international reference laboratory be designated in order to facilitate these goals, and to establish a collection of characterised equine influenza virus strains.

5. Standards for production, potency, safety and vaccination schedules be worked out since present regulations concerning vaccine standards and vaccination schedules are inadequate, and that a task force should be set up to organise a cooperative vaccine trial.

6. In the case of an outbreak of equine influenza, the international reference laboratory informs relevant organisations, specialists and vaccine producers about the clinical and epizootiological situation and on properties of virus isolates from that outbreak.

7. It be realised that the only possible way to interrupt dissemination of a new virus subtype (as evidenced first by severe clinical symptoms in horses of all ages) is to restrict movement of horses without delay.
8. The horse racing authorities should take advantage of the resources available to carry out the recommendations of this Group of Experts.