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# Isolation of an equine influenza virus strain and epizootiological study of the 1985-86 outbreak in Argentina

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*Summary: During an outbreak of equine influenza in Argentina in the summer of 1985-86, most of the country's horses were affected. Equine influenza virus isolated from 6 of 20 sampled animals proved to be an A/equi 2 subtype. The isolated strain was named A/equi 2/La Plata 85. An epizootiological study was conducted on 99 out of the 3,000 horses at La Plata Racecourse. Most serum samples taken in the acute stage were negative against both the La Plata and the prototype Miami 63 strains. 98.8% of convalescent horses had positive antibody titres against the La Plata isolate and 71.7% had positive antibody titres against Miami strain. Serum samples obtained six months after infection showed 51.5% and 9% of positive titres against La Plata and Miami strains, respectively.*

KEYWORDS: Antibodies - Argentina - Equine influenza - Horse diseases - Influenzavirus.

## INTRODUCTION

Equine influenza is an acute respiratory tract disease of horses. The onset of clinical signs is sudden after an incubation period of 3 to 5 days. It is manifested by high fever, anorexia, depression and a dry deep cough which is characteristic of the disease.

Equine influenza may be caused by type H7N7 (A/equi 1 subtype) or type H3N8 (A/equi 2 subtype) virus. The former is represented by the A/equi 1/Prague 56 strain (2) and the latter by the A/equi 2/Miami 63 strain (3).

An outbreak of influenza in horses in Argentina caused by an A/equi 1 strain was described in 1976 (1). The second epizootic occurred during the summer of 1985-86. The virus spread rapidly throughout the country and most horses became

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ill. The purpose of this paper is to report the isolation of a new influenza A/equi 2 strain and to present a survey of this outbreak among 3,000 horses in La Plata Racecourse, Buenos Aires Province.

## MATERIALS AND METHODS

### Samples

Nasal swabs, blood mixed with 10% EDTA solution (2% EDTA in saline solution) and fecal samples were collected from 20 horses for virus isolation. A total of 99 serum samples were obtained from these and another 79 acutely ill horses. Serum samples were obtained from the same 99 horses during convalescence and six months after infection. They were stored at $-20°C$. Monospecific sera were prepared against each strain in chickens.

All the horses had been vaccinated twice with an inactivated influenza vaccine (Miami and Prague strains) two months before and during the outbreak.

### Virus isolation

Nasal swabs, plasma and fecal samples were inoculated into equine fetal kidney primary cell cultures and VERO, BHK and MA-104 cell lines. Nasal swab samples were also inoculated into 10-day-old embryonated chicken eggs by the amniotic and allantoic routes.

Cell cultures were examined for the presence of cytopathic effect (CPE). Those cultures which remained negative after seven days received two additional passages before being considered negative. The eggs were incubated at 37°C for 72 hours. After chilling, the allantoic and amniotic fluids were harvested and tested for haemagglutinating activity using 0.5% suspensions of chicken erythrocytes. Two additional passages by the allantoic route were made if the fluids showed negative results. All isolates were identified using the haemagglutination-inhibition (HI) test with specific antisera against H7N7 and H3N8 influenza virus strains, supplied by Dr J.T. Bryans, Kentucky, USA.

### HI test

This test was performed in a microsystem according to conventional methods. Sera were treated with 1/90 M $KIO_4$ (periodate) at room temperature for 60 minutes. Periodate was then inhibited with 1% glycerin solution in saline (0.85% NaCl) for 60 minutes, then at 56°C for 30 minutes and was finally absorbed with chicken erythrocytes. The final test serum was a 1:8 dilution of the serum collected in saline solution. A/equi 1/Prague 56 (H7N7), A/equi 2/Miami 63 (H3N8) and the newly isolated strains were used as antigens.

Tests were performed in round-bottomed microtitre plates using volumes of 0.025 ml. Two-fold dilutions of serum in saline solution with 0.1% gelatine were prepared, eight haemagglutinating units of antigen were added and the mixtures were allowed to interact for 60 minutes at room temperature before adding 0.5% chicken erythrocytes. Results were recorded after 60 minutes and HI titres were expressed as reciprocals.

## RESULTS

More than 90% of a total of 3,000 horses showed clinical signs of the disease. No differences related to sex and age were seen.

No apparent CPE was observed in inoculated cell cultures after three passages.

Haemagglutinating agents were recovered from embryonated eggs after one passage (with a mean titre of 1:64) from 6 of 20 sampled animals. Titres increased to 1:512 after the third passage.

The isolated virus and the homologous antiserum prepared in chickens were assayed against Miami 63 and Prague 56 viral strains and the respective antisera by HI tests. Results were negative against the Prague strain. The HI titre of the isolate against the homologous antiserum was 512, and against the anti-Miami serum it was 64, while the titre of the same homologous antiserum against the Miami strain was 128 (Table I).

Paired serum samples were obtained from 20 horses during the acute phase of the disease and again two weeks later. Specific antibodies were detected by HI tests in sera from 5 sick animals. All recovered horses had high titres of specific antibodies.

**TABLE I**

*Cross-reaction between the isolated virus, two reference strains and their respective antisera*

| | Virus | | |
|---|---|---|---|
| **Antisera** | **Prague** | **Miami** | **La Plata** |
| Prague | 128[a] | – | – |
| Miami | – | 256 | 64 |
| La Plata | – | 128 | 512 |

(a) Reciprocal of HI titres

### Epidemiological study

Twenty sera collected previously and three sets of 99 sera obtained during the acute phase (1st samples), the convalescent phase (2nd samples) and six months afterwards (3rd samples) were examined by HI test using Prague 56, Miami 63 and the isolated strains. Among the 1st samples 15%, 10% and 5% sera showed positive titres against Prague, Miami and the isolated strains, respectively. Of the 2nd samples 23% had antibody titres against Prague, 71.7% against Miami and 98.9% against the isolated virus. None of the 3rd samples were positive against Prague strain, but 9% and 51.5% of them were positive against Miami and the isolated strains, respectively (Table II).

The antibody titres against Miami and the isolated strains were low in the 1st samples, very high (up to 512) in the 2nd samples and had decreased in the 3rd samples

**TABLE II**

*Antibody titres against different equine influenza virus strains*

| HI titres | Virus strains: Prague 1S* | Prague 2S | Prague 3S | Miami 1S | Miami 2S | Miami 3S | La Plata 1S | La Plata 2S | La Plata 3S |
|---|---|---|---|---|---|---|---|---|---|
| < 8 | 84** | 76 | 99 | 89 | 28 | 90 | 94 | 1 | 48 |
| 8 | 9 | 7 | | 3 | 7 | 5 | 3 | 9 | 23 |
| 16 | 3 | 8 | | 1 | 16 | 4 | 1 | 14 | 21 |
| 32 | 3 | 8 | | 4 | 17 | | 1 | 22 | 6 |
| 64 | | | | 2 | 12 | | | 20 | 1 |
| 128 | | | | | 8 | | | 15 | |
| 256 | | | | | 10 | | | 16 | |
| 512 | | | | | 1 | | | 2 | |
| Positive (%) | 15 | 23 | 0 | 10 | 71.7 | 9 | 5 | 98.9 | 51.5 |

* 1S: 1st sample obtained during the acute phase of the disease
2S: 2nd sample obtained during the convalescent period (after 2 weeks)
3S: 3rd sample obtained after 6 months

** Positive/total 99 sera

(Table II). Mean titres, expressed as geometric means (log 2), were low against Prague strain. In the 2nd and 3rd samples the mean titres of the isolated strain were higher than those of the Miami strain (Table III).

**TABLE III**

*Mean of antibody titres against different equine influenza virus strains**

| Virus | Sera 1S** | Sera 2S | Sera 3S |
|---|---|---|---|
| Prague | 1.6 | 2.04 | 0 |
| Miami | 2.5 | 3.45 | 1.44 |
| La Plata | 1.6 | 3.76 | 3.16 |

* Geometric mean (log 2)
** 1S: 1st sample obtained during the acute phase of the disease
2S: 2nd sample obtained during the convalescent period
3S: 3rd sample obtained after 6 months

## DISCUSSION

The clinical and epidemiological pictures were consistent with equine influenza. More than 90% of 3,000 horses showed clinical signs of the disease at La Plata Racecourse. The entire equine population had been vaccinated twice with an inactivated influenza vaccine containing Miami and Prague strains two months before

and during the outbreak. Attempts were made to isolate a virus other than equine influenza virus in monolayer cell cultures but the results were negative. An HA-positive agent was, however, recovered from the inoculated eggs. The isolate and two reference strains were tested against three different specific antisera (Table I). Considering that the titres against Miami strain were lower than those of the isolated virus, it seemed that there may have been antigenic drift away from the prototype strain. The isolated virus was named A/equi 2/La Plata 85 strain.

Serological studies were carried out on 99 serum samples.

Regarding the A/equi 1/Prague 56 strain, there were a few positive titres (15%) in the samples obtained during the acute period, with a geometric mean titre of 1.6. The positive titres were 23% during the convalescent period, and although the geometric mean titre was slightly higher (2.04), it was still relatively low.

It was assumed that this titre increase was due to vaccination of the horses during the outbreak.

As far as A/equi 2/Miami 63 strain is concerned, 10% of the samples were positive in the acute phase, and two weeks later the figure rose to 71.7% (geometric means of 2.5 and 3.45, respectively). High titres may be due to cross-reaction between the vaccinal strains and the La Plata strain. This was indicated by the sharp decrease observed in the number of positive animals (9%) and in the antibody titres (geometric mean titre of 1.44) in the third samples.

With regard to A/equi 2/La Plata 85 strain, only 5% of the horses were positive in the acute phase. This indicates that most samples had been taken before antibody formation had commenced, and that the low titres were due to cross-reaction with antibodies against vaccine strains. Nearly all convalescent horses were positive, with titres ranging from 32 to 512 (geometric mean 3.76). The sera obtained six months afterwards were positive in 51% of cases with a geometric mean titre of 3.16.

It may be concluded that specific antibodies against La Plata strain persisted for a long time. This means that La Plata strain was the cause of the outbreak, and that it differs slightly from the prototype Miami strain.

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**ISOLEMENT D'UNE SOUCHE DU VIRUS DE LA GRIPPE ÉQUINE ET ÉTUDE ÉPIZOOTIOLOGIQUE DE L'ÉPIZOOTIE DE 1985-1986 EN ARGENTINE. – E. Nosetto, M. Pecoraro, C.M. Galosi, R. Massone, V. Cid de la Paz, R. Ando, Y. Ando et M.E. Etcheverrigaray.**

***Résumé :*** *Au cours d'une épizootie de grippe équine qui a évolué en Argentine au cours de l'été 1985-86, la plupart des chevaux du pays ont été atteints. Le virus de la grippe équine isolé à partir de 6 chevaux sur 20 ayant fait l'objet*

*de prélèvements, s'est avéré appartenir au sous-type A/equi 2. La souche isolée a été désignée A/equi 2/La Plata 85. Une étude épizootiologique a été effectuée sur 99 des 3 000 chevaux de l'Hippodrome de La Plata. La plupart des prélèvements de sérums recueillis au stade aigu de la maladie se sont montrés négatifs à la fois vis-à-vis de la souche La Plata et de la souche prototype Miami 63. 98,8 % des chevaux convalescents avaient des titres d'anticorps positifs vis-à-vis de la souche La Plata, et 71,7 % vis-à-vis de la souche Miami. Parmi les prélèvements de sérum recueillis six mois après l'infection, 51,5 % avaient des titres positifs vis-à-vis de la souche La Plata et 9 % vis-à-vis de la souche Miami.*

MOTS-CLÉS : Anticorps - Argentine - Grippe équine - Maladies des chevaux - Virus influenza.

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**AISLAMIENTO DE UNA CEPA DEL VIRUS DE LA INFLUENZA EQUINA Y ESTUDIO EPIZOOTIOLÓGICO DE LA EPIZOOTIA DE 1985-1986 EN ARGENTINA. – E. Nosetto, M. Pecoraro, C.M. Galosi, R. Massone, V. Cid de la Paz, R. Ando, Y. Ando y M.E. Etcheverrigaray.**

***Resumen:*** *Se estudió una epizootia de influenza equina en Argentina. La mayoría de los caballos del país sufrieron la infección en el verano de 1985-86. Se tomaron muestras de 20 animales aislándose el virus de influenza equina de 6 de ellos. La cepa viral aislada demostró ser un subtipo A/equi 2 y fue denominada A/equi 2/La Plata 85. Se realizó un estudio epizootiológico en 99 caballos sobre una población aproximada de 3.000 animales del Hipódromo de La Plata. La mayoría de las muestras de suero sanguíneo tomadas en el período agudo de la enfermedad fueron negativas contra la cepa La Plata y la cepa prototipo Miami 63. El 98,8% de los animales convalescientes desarrollaron anticuerpos contra el virus aislado y 71,7% contra la cepa Miami. Las muestras tomadas a los 6 meses post infección mostraron 51,5% y 9% de positivos contra las cepas La Plata y Miami respectivamente.*

PALABRAS CLAVE: Anticuerpos - Argentina - Enfermedades de los caballos - Influenza equina - Influenza virus.

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