



*Havemeyer Foundation  
Monograph Series No. 7*

*Proceedings of the*

**FOURTH INTERNATIONAL MEETING  
OF OIE AND WHO EXPERTS ON  
CONTROL OF EQUINE INFLUENZA**

*Surveillance and vaccine efficacy:  
the American perspective*

*3rd-5th August 1999  
Miami, Florida, USA*

**Editors: J. A. Mumford, J. M. Daly and J. F. Wade**



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## FOREWORD

This was the fourth in a series of consultative meetings initiated by WHO as part of their programme on the ecology of equine influenza viruses. The common aim has been to improve our understanding of the epidemiology and control of the disease at an international level.

The previous meetings, in 1983, 1992 and 1995, were held in Europe. However, this one focused particularly on the American perspective, in view of the extensive and very mobile equine population within the Americas and the dynamic export market. The choice of Miami as a venue was based on practical considerations and reflected our desire to involve all interested parties, from the Americas and the Caribbean.

We used the occasion to bring together representatives of the veterinary, regulatory, scientific, equine and commercial sectors. It was felt that rapid progress could be achieved only by providing a forum for discussion and collaboration between these groups. We were aware of significant variation, between countries, with regard to vaccine efficacy, enforced vaccination/quarantine procedures and diagnostic capabilities. In the face of ever-increasing movement of horses around the world, it is essential to secure greater international harmonisation and leadership from America in this regard would be of great benefit.

The specific aims of the meeting were:

- To generate an interest in, and support for, improved diagnosis, virus isolation, surveillance and reporting of equine influenza;
- To review the activities of the Equine Influenza Expert Surveillance Panel and assess the criteria for vaccine strain selection;

- To evaluate the performance of vaccines currently available in the Americas and examine available standards for vaccine licensing;
- To address regulatory issues relating to the introduction of a fast track licensing system when vaccine strains are updated;
- To initiate activities aimed at achieving international harmonisation of vaccine standards to support control programmes;
- To examine the role of vaccination in codes of practice and regulations relating to control of influenza and international movement of horses.

The series of meetings, and the activities of the Equine Influenza Expert Surveillance Panel, are of paramount importance to the international horse industry. The rise in global transport among horses represents a correspondingly high risk of disease spread and constant monitoring is, therefore, essential.

This meeting was made possible by the generosity of the Dorothy L Russell Havemeyer Foundation and I am extremely grateful to Mr Gene Pranzo for his foresight, in agreeing to fund the meeting, and his patience in relation to the unavoidable delays in bringing these proceedings to fruition. I would also like to thank the Horserace Betting Levy Board, Bayer Corporation Animal Health, Boehringer Ingelheim Animal Health Inc, Fort Dodge Animal Health, Heska Corporation, Hoechst Roussel Vet, Intervet International bv, Merial, Schering-Plough Animal Health, all of whom gave the meeting their support.

*Dr Jenny Mumford*  
*Chairman*

## ABBREVIATIONS

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<b>ANOVA</b>	analysis of variance
<b>C</b>	celsius/cough
<b>CCA</b>	chick cell agglutination
<b>EC</b>	European Commission
<b>EIA</b>	equine infectious anaemia
<b>Eu Ph</b>	European Pharmacopoeia
<b>EU</b>	European Union
<b>ELISA</b>	enzyme-linked immunosorbent assay
<b>GMT</b>	geometric mean titre
<b>HA</b>	haemagglutinin
<b>HI</b>	haemagglutination inhibition
<b>ICC</b>	International Collating Centre
<b>IURD</b>	infectious upper respiratory tract disease
<b>ISCOM</b>	immune stimulating complex
<b>MDCK</b>	Madin-Darby canine kidney
<b>mod.</b>	moderate
<b>NAHMS</b>	National Animal Health Monitoring System (USA)
<b>NASS</b>	National Agricultural Statistics Service (USA)
<b>ND</b>	nasal discharge
<b>NH</b>	national hunt
<b>NIBSC</b>	National Institute for Biological Standards and Control
<b>OIE</b>	Office International des Epizooties
<b>P</b>	probability/pyrexia
<b>P-P</b>	point-to-point
<b>RAC</b>	relative antibody concentration
<b>RT/PCR</b>	reverse transcription/polymerase chain reaction
<b>s/c</b>	seroconversion
<b>SRD</b>	single radial diffusion
<b>SRH</b>	single radial haemolysis
<b>TB</b>	Thoroughbred
<b>WHO</b>	World Health Organization

# HAVEMEYER SCIENTIFIC WORKSHOPS

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- 1981            **First International Workshop on Lymphocyte Alloantigens of the Horse**  
October - New York City, USA  
*Organiser: Dr D. F. Antczak*
- 1982            **Second International Workshop on Lymphocyte Alloantigens of the Horse**  
October - Cornell University, Ithaca, New York, USA  
*Organiser: Dr D. F. Antczak*
- 1983            **Third International Workshop on Lymphocyte Alloantigens of the Horse**  
April - New Bolton Center, University of Pennsylvania, USA  
*Organiser: Dr D. F. Antczak*
- 1984            **First International Symposium on Equine Embryo Transfer**  
October - Cornell University, Ithaca, New York, USA  
*Organisers: Drs D. F. Antczak and W. R. Allen*
- 1985            **Fourth International Workshop on Lymphocyte Alloantigens of the Horse**  
October - University of Kentucky, USA  
*Organisers: Drs D. F. Antczak and E. Bailey*
- 1986            **Workshop on *Corynebacterium equi* Pneumonia of Foals**  
July - University of Guelph, Canada  
*Organiser: Dr J. F. Prescott*
- 1987            **Fifth International Workshop on Lymphocyte Alloantigens of the Horse**  
October - Louisiana State University, USA  
*Organisers: Drs D. F. Antczak and J. McClure*
- 1989            **Second International Symposium on Equine Embryo Transfer**  
February - Banff, Alberta, Canada  
*Organisers: Drs D. F. Antczak and W. R. Allen*
- 1990            **International Workshop on Equine Sarcoids**  
April - Interlaken, Switzerland  
*Organisers: Dr D. F. Antczak and Professor S. Lazary*
- 1992            **Workshop on Equine Neonatal Medicine**  
January - Naples, Florida  
*Organisers: Drs D. F. Antczak and P. D. Rossdale*
- Third International Symposium on Equine Embryo Transfer**  
February - Buenos Aires, Argentina  
*Organisers: Drs D. F. Antczak, W. R. Allen, J. G. Oriol and R. Pashen*

- 1995
- Equine Perinatology**  
July - Cambridge, England  
*Organiser: Dr P. D. Rossdale*
- Second International Equine Leucocyte Antigen Workshop**  
July - Lake Tahoe, California, USA  
*Organisers: Drs D. F. Antczak, P. Lunn and M. Holmes*
- First International Workshop on Equine Gene Mapping**  
October - Lexington, Kentucky, USA  
*Organisers: Drs D. F. Antczak and E. Bailey*
- Erection and Ejaculation in the Human Male and Stallion: A Comparative Study**  
October - Mount Joy, Pennsylvania, USA  
*Organiser: Dr S. M. McDonnell*
- Bone Remodelling Workshop**  
October - Corcord, Massachusetts, USA  
*Organiser: Dr H. Seeherman*
- 1997
- Second International Workshop on Equine Gene Mapping**  
October - San Diego, California, USA  
*Organisers: Drs D. F. Antczak and E. Bailey*
- Maternal Recognition of Pregnancy in the Mare**  
January - Dominican Republic  
*Organisers: Drs W. R. Allen and T. A. E. Stout*
- Uterine Clearance**  
March - Gainesville, Florida, USA  
*Organiser: Dr M. M. LeBlanc*
- Trophoblast Differentiation**  
September - Edinburgh, Scotland  
*Organisers: Drs D. F. Antczak and F. Stewart*
- 1998
- Third International Genome Workshop**  
January - San Diego, California, USA  
*Organisers: Drs D. F. Antczak and E. Bailey*
- Third International Workshop on Perinatology: Genesis and Post Natal Consequences of Abnormal Intrauterine Developments: Comparative Aspects**  
February - Sydney, Australia  
*Organiser: Dr P. D. Rossdale*
- Horse Genomics and the Genetic Factors Affecting Race Horse Performance**  
March - Banbury Center, Cold Spring Harbor, New York, USA  
*Organisers: Drs D. F. Antczak, E. Bailey and J. Witkowski*

**Allergic Diseases of the Horse**

April - Lipica, Slovenia

*Organisers: Drs D. F. Antczak, S. Lazary and E. Marti*

**Equine Placentitis Workshop**

October - Lexington, Kentucky, USA

*Organisers: Drs D. F. Antczak, W. R. Allen and W. Zent*

**Septicemia II Workshop**

November - Boston, Massachusetts, USA

*Organiser: Dr M. R. Paradis*

1999

**Equine Genome Project**

January - San Diego, California, USA

*Organisers: Drs D. F. Antczak and E. Bailey*

**Third International Equine Genome Workshop**

June - Uppsala, Sweden

*Organisers: Drs D. F. Antczak, E. Bailey and K. Sandberg*

**Fourth International Meeting of OIE and WHO Experts on Control of Equine Influenza**

August - Miami, Florida, USA

*Organiser: Dr J. Mumford*

**European Equine Gamete Workshop**

September - Lopuszna, Poland

*Organisers: Drs W. R. Allen and M. Tischner*

**Fetomaternal Control of Pregnancy**

November - Barbados, West Indies

*Organisers: Drs T. Stout and W. R. Allen*

2000

**Equine Genome Project**

January - San Diego, California, USA

*Organisers: Drs D. F. Antczak and E. Bailey*

**Uterine Infections in Mares and Women: A Comparative Study**

March - Naples, Florida, USA

*Organiser: Dr M. M. LeBlanc*

**5th International Symposium on Equine Embryo Transfer**

July - Saari, Finland

*Organiser: Dr T. Katila*

2001

**USDA International Plant & Animal Genome Conference**

January - San Diego, California

**Equine Immunology in 2001**

January - Santa Fe, New Mexico

*Organiser: Dr D. P. Lunn*

**Asthma and Allergies II**

April - Hungary

*Organisers: S. Lazary and E. Marti*

**From Elephants to Aids**

June - Port Douglas, Australia

*Organiser: Professor W. R. Allen*

**International Equine Gene Mapping**

July - Brisbane, Australia

*Organiser: K. Bell*

**Second Meeting of the European Gamete Group (EEGG)**

September - Loosdrecht, The Netherlands

*Organiser: Dr T. A. E. Stout*

**Foal Septicemia III**

October - Tufts University European Center, Talloires, France

*Organiser: M. R. Paradis*

**Infectious Disease Programme for the Equine Industry and Veterinary Practitioners**

October - Marilyn duPont Scott Medical Center, Morvan Park, Virginia, USA

*Organisers: Drs J. A. Mumford and F. Fregin*

**From Epididymis to Embryo**

October - Fairmont Hotel, New Orleans, USA

*Organiser: Dr L. H-A. Morris*

2002

**USDA International Plant & Animal Genome Conference**

January - San Diego, California

**Comparative Neonatology/Perinatology**

January - Palm Springs, California

*Organiser: P. Sibbons*

**Stallion Behavior IV**

June - Reykjavik, Iceland

*Organisers: S. McDonell and D. Miller*

**Rhodococcus Equi II**

July - Pullman, Washington

*Organiser: J. Prescott*

**Equine Orthopaedic Infection**

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# SESSION I:

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## Epidemiology of equine influenza

*Chairman: J. A. Mumford*

## OVERVIEW OF EQUINE INFLUENZA FROM THE AMERICAN PERSPECTIVE

D. G. Powell

The American perspective was introduced with a summary of the historical incidence of equine influenza in the USA between 1960 and 1979 (Fig 1). Prior to 1963, H7N7 subtype viruses caused outbreaks of influenza in horses and the last of these in the USA occurred in Kentucky in 1977. In 1963, although sporadic outbreaks continued to be caused by H7N7 subtype viruses, a novel H3N8 strain was the cause of a continental and international epizootic. The movement of horses from South America was the most probable source of these outbreaks, the first of which occurred in Miami in January 1963. The subsequent itinerant movement of racing Standardbred and Thoroughbred horses in the first half of the year led to widespread dissemination of disease along both the eastern and western seaboard of the USA and eventually into Canada (Fig 2).

Between 1980 and 1999 there continued to be sporadic outbreaks of disease caused by H3N8 subtype viruses, particularly in the eastern states but also in Colorado, California and Alaska. During this period, there were only 4 years in which influenza has not been diagnosed such that equine influenza is now generally considered to be an endemic disease of North America (Fig 3).

The importance of the demographic structure of the equine population to the occurrence and endemic nature of influenza in the USA was demonstrated. A survey by the American Horse Council in 1996 put the total US horse population at 6.9 million, the equivalent figure from the National Agricultural Statistics Service (NASS) in

1999 was 5.25 million horses (Fig 4). The NASS survey also showed the largest populations occurred in Texas, where no influenza is reported, and California, which reports the disease only sporadically.

Detailed breakdown of the demography by the National Animal Health Monitoring System (NAHMS) in 1998 showed that the predominant breed was Quarter horses (Table 1a), and the predominant type was pleasure/recreational animals (Table 1b). These data showed that most horse operations comprised 5 or less horses (Table 1c) and this undoubtedly presented difficulties with obtaining samples, which were more likely to come from larger operations as found typically in Kentucky. The NAHMS survey showed that 40% of premises did not vaccinate resident horses.

Lastly the movement of horses in and out of the USA was put in the context of numbers of horses being imported and exported. Figures from the National Center for Import and Export showed that 72,500 horses were imported in 1997/98 (Fig 5), and 90,000 horses were exported for the same period with over 80% of this movement being conducted with Canada (Fig 6).

This large movement of horses has had an important impact on the spread of equine influenza, with the USA playing an apparently increasing role in the spread of the disease to other parts of the world (Table 2). This emphasises the increasing importance of molecular techniques in the tracking of the global spread of viral isolates.

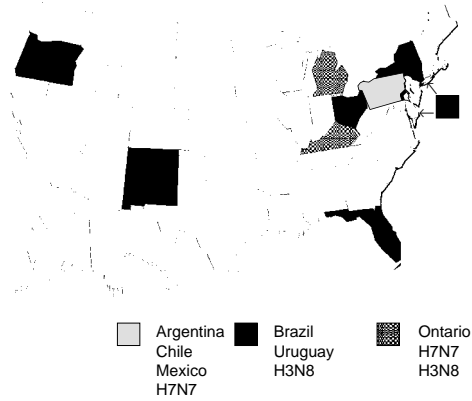


Fig 1: Equine influenza in the USA between 1960 and 1979.

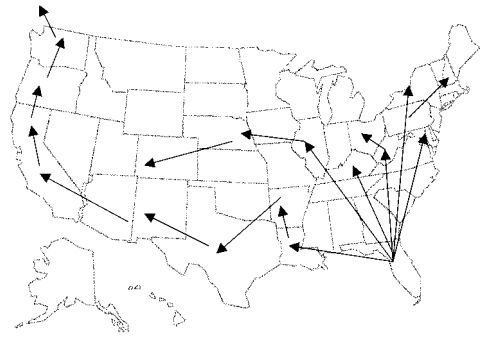
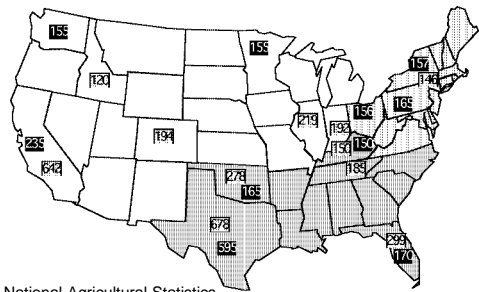


Fig 2: Equine influenza epizootic dissemination, 1963 (courtesy of Ralph Knowles).



Fig 3: Equine influenza in the USA between 1980 and 1999.



National Agricultural Statistics Services (NASS), 1999  
 Total population = 5,250  
 (White numbers)  
 Central state = 883  
 North eastern state = 522  
 Southern states = 719  
 Western states = 905

American Horse Council  
 Baren's Group, 1995  
 Total population = 6,900  
 (black numbers)

Fig 4: US equine populations (thousand head).

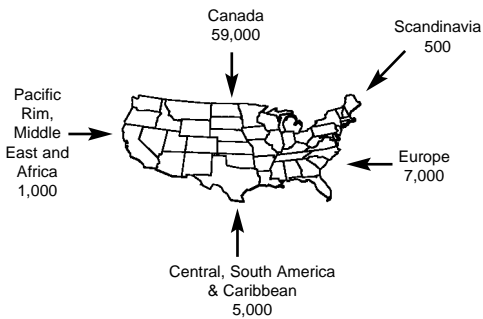


Fig 5: Equine imports into the USA between 1997 and 1998 (total 72,500).

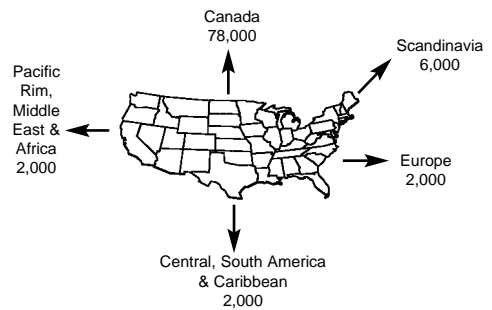


Fig 6: Equine exports from the USA, 1998 (total 90,000).

**TABLE 1: Equine demographics in the USA (Source: Equine 98 National Animal Health Monitoring System, USDA:APHIS:VS, August 1998)**

a) Breed	%		
Appaloosa	5.3		
Arabian	4.5		
Draft	1.1		
Paint	4.7		
Standardbred	2.1		
Tennessee bred	8.2		
Thoroughbred	14.2		
Quarter horse	42.1		
Other (reg.)	9.2		
Other (not reg.)	8.6		
b) Primary use	%		
Pleasure	66.8		
Showing/competition	6.5		
Breeding	6.0		
Racing	1.9		
Farm/ranch	15.2		
Other	3.6		
c)	Size of operation (%)		
	1-5	6-19	20
Operation	78	18	4
Equids	40	33	27

**TABLE 2: Outbreaks of equine influenza in the USA resulting from international movement of equids**

Virus subtype	Importing country	Year	Source
H3N8	USA	1963	S. America
H3N8	Western Europe	1965	N. America
H7N7	UK, Ireland	1977	Europe
H7N7	Singapore, Malaysia	1977	UK
H3N8	UK, Ireland	1979	Europe
H3N8	South Africa	1986	USA
H3N8	India	1987	France
H3N8	Jamaica	1989	USA
H3N8	Hong Kong	1992	UK
H3N8	UAE (Dubai)	1995/6	USA
H3N8	Puerto Rico	1997	USA
H3N8	Philippines	1997	USA

## INTERNATIONAL COLLATING CENTRE REPORT

J. R. Newton

The International Collating Centre (ICC) was initially conceived at the International Breeders' Meeting (IBM) held in California in October 1986 and was established at the Animal Health Trust, Newmarket, England, in January 1987. Membership is open to both member and non-member IBM countries. Participating countries nominate a veterinary contact to be responsible for reporting episodes of infectious disease from their country on a quarterly basis. In turn, the ICC provides quarterly disease reports, and supplementary reports as necessary, to subscribers worldwide.

The data collated by the ICC are limited to participating countries and comes from only one contact within countries and may therefore be fairly regional and incomplete. However, the data are the only type of their kind for non-notifiable

diseases such as equine influenza and they do provide a unique global perspective. Inclusion of influenza in quarterly reports requires that a laboratory diagnosis based on serology, virus isolation or nucleoprotein ELISA is made. Clinical evidence of influenza alone is not an acceptable criterion for diagnosis. Table 3 summarises the collated reports for diagnosis of equine influenza made by ICC subscribing countries for 1995 to 1997 and Table 4 summarises similar data for 1998 and the first 2 quarters of 1999. The data show that during this period in Europe the majority of influenza diagnoses were made in France, Sweden and the UK; in North America most diagnoses were notified from Kentucky and, since 1995, there have been no notifications of influenza in subscribing countries in Africa, Asia and Australasia.

**TABLE 3: Notifications of influenza diagnoses reported to the International Collating Centre, Newmarket from 1995 to 1997**

Country	Continent	Quarters – 1995				Quarters – 1996				Quarters – 1997			
		1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
South Africa	Africa												
Hong Kong	Asia												
Japan	Asia					NR							
Singapore	Asia												
Australia	Australasia												
New Zealand	Australasia							NR					
<b>Belgium</b>	<b>Europe</b>										NR	NR	NR
<b>Denmark</b>	<b>Europe</b>		NR			NR	✓					✓	
France	Europe	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Germany	Europe	NR											
<b>Ireland</b>	<b>Europe</b>	✓									✓		NR
<b>Italy</b>	<b>Europe</b>							✓					
Netherlands	Europe				NR		NR		NR		NR		NR
<b>Norway</b>	<b>Europe</b>	NR	NR	NR	NR			NR	NR	✓	✓		
<b>Sweden</b>	<b>Europe</b>	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	
<b>Switzerland</b>	<b>Europe</b>		✓	✓	✓								
<b>UK</b>	<b>Europe</b>	✓	✓	✓	✓		✓	✓			✓	✓	✓
<b>UAE</b>	<b>Middle East</b>					✓							
Canada	N America	NR	NR		NR					NR	NR	NR	NR
<b>USA</b>	<b>N America</b>	✓KY		✓KY					✓KY			✓KY	✓CA

✓ = influenza diagnosed in the quarter; NR = no quarterly report received from that country; KY = Kentucky; CA = California

**TABLE 4: Notifications of influenza diagnoses reported to the International Collating Centre, Newmarket for 1998 and 1999**

Country	Continent	Quarters –1998				Quarters – 1999	
		1st	2nd	3rd	4th	1st	2nd
South Africa	Africa						
Hong Kong	Asia						
Japan	Asia				NR		
Singapore	Asia						
Australia	Australasia						
New Zealand	Australasia						
<b>Denmark</b>	<b>Europe</b>				✓		
<b>France</b>	<b>Europe</b>		✓	✓	✓	✓	✓
Germany	Europe			NR		NR	
<b>Ireland</b>	<b>Europe</b>		✓				
Italy	Europe						
Netherlands	Europe						NR
Norway	Europe				NR		
<b>Sweden</b>	<b>Europe</b>		✓	✓		✓	✓
Switzerland	Europe					✓	✓
<b>UK</b>	<b>Europe</b>		✓	✓		✓	✓
UAE	Middle East						
Canada	N America						
<b>USA</b>	<b>N America</b>			✓KY			✓KY

✓ = Influenza diagnosed in the quarter; NR = no quarterly report received from that country; KY = Kentucky

## REGIONAL REPORTS OF OUTBREAKS DIAGNOSED AND DOMESTIC VACCINATION POLICIES

### NORTH AMERICA

#### *Canada (H. Townsend)*

There are no current ongoing formal surveillance programmes in place in Canada. Outbreaks of respiratory disease are not investigated routinely to determine the aetiology, particularly those that appear to be due to viral infections. However, outbreaks of upper respiratory tract disease are considered to occur commonly and, based on previous studies, it is assumed that many of these are due to influenza infections. As part of a vaccine field trial performed in the summer of 1998 to compare serological response of horses to vaccines marketed in Canada, serum samples were collected from 108 horses in and around the city of Saskatoon. None of these horses had a record of vaccination in the past 12 months; 94% were considered seropositive prior to initiation of the vaccine trial and 34% had serum antibody concentrations consistent with clinical protection. The horses came from 4 properties and all horses in the study had frequent direct or indirect contact with animals from other properties. These observations are consistent with the view that influenza is endemic in the Canadian horse population. In contrast, it is known that serum samples from many large horse operations in western Canada that have limited contact with other facilities and do not vaccinate against the disease, do not contain antibodies to influenza. With the exception of certain events such as the Pan-American Games, recently held in Winnipeg, Manitoba, there are no compulsory influenza vaccination programmes in Canada and there is no general requirement for horses entering or leaving the country to be vaccinated.

#### *New York State (A. Glaser)*

Equine influenza infection has only been confirmed 3 times in the last 18 months by the New York State Diagnostic Laboratory. Seroconversions occurred in 2 horses from Ohio and New York State and a third horse, from an 'outbreak of respiratory disease' also in New York State, was diagnosed by isolation of an H3N8 virus from a nasal swab. Part of the failure to identify influenza outbreaks may be

due to the submission of only single serum samples and the paucity of diagnostic samples from acutely affected horses submitted for virus isolation. This may be due to the relatively high cost of virus isolation and its perceived lack of benefit. The confirmation of influenza at 4–6 month intervals over the last 18 months suggests that the virus is actively circulating. However, more efficient surveillance will require the co-operation of clinicians, diagnostic laboratories and would benefit from the availability of a central subsidised laboratory to identify and characterise viral isolates. There is no formal policy regarding influenza vaccination in New York State and as such it remains voluntary. As a result, the horses being vaccinated routinely are the higher value performance horses.

#### *Colorado (J. Traub-Dargatz)*

A study to monitor acute viral respiratory tract disease in horses in Colorado was conducted in 1996. Cases of infectious upper respiratory disease were notified to Colorado State University (CSU) from regional veterinarians. At an initial visit a health and management history was taken and serum and whole blood samples and a nasal/nasopharyngeal swab were taken. A convalescent visit was conducted 12–14 days later when a second serum sample was collected. Laboratory tests included Directigen Flu-A ELISA and virus isolation performed on swabs and haemagglutination inhibition (HI) titres were determined to 3 strains of influenza virus, A/eq/Prague/56 (H7N7), A/eq/Miami/63 (H3N8) and A/eq/Kentucky/81 (H3N8). Samples were collected from 112 acutely ill horses on 56 premises and of these, 47 (42%) were diagnosed with influenza. Results showed that there was no significant difference in the vaccination history between acutely ill horses diagnosed with influenza and those not diagnosed with the infection. Twenty-three of 34 (68%) horses with influenza had been vaccinated in the 3 months prior to becoming ill. The majority of sick horses that were aged 4 years or less had HI titres of 1/16 or less and of 21 horses tested positive by Directigen Flu-A assay, 18 (86%) had HI titres to

H3N8 virus of less than 1/8. In a continuation of this study in the 16 months between June 1997 and October 1998, 6 premises were monitored in northern Colorado. Influenza was diagnosed on one premise in December 1997 with seroconversions demonstrated to both H7N7 and H3N8 viruses and a positive Directigen Flu-A test. In January 1999, influenza was diagnosed at the CSU veterinary teaching hospital in Fort Collins. Among the population of 40 horses, 7 were sampled of which 2 were Directigen test positive and virus was isolated.

**Ohio (P. Morley)**

In investigations of infectious upper respiratory disease (IURD) outbreaks between June 1998 and May 1999, Ohio State University confirmed infection with influenza virus on 6 premises, and the details of these investigations are presented below in Table 5.

**Kentucky (T. Chambers)**

For the period between 1995 and 1999 there was no evidence of influenza caused by H7N7 virus infection but H3N8 viruses were isolated every year. Outbreaks were typically sporadic and on a small scale with generally less than 10 horses being affected. Yearlings, 2- and 3-year-old horses have been affected with signs of transient severe fever, lethargy, cough and nasal discharge. Influenza has occurred in horses on both breeding farms and at racetracks. Same day diagnosis has been accomplished using the Directigen Flu-A test. A new rapid test, Z-statflu, developed for detection of A and B strains in humans was found to detect both H7N7 and H3N8 isolates but was more cumbersome and not as useful as the Directigen test. There is anecdotal evidence that there has been interstate transmission of influenza to and from Kentucky but precise data are not available.

Poor communication prevented sampling of some suspected outbreaks and many veterinarians were reluctant to take nasopharyngeal swabs as they perceived no benefit in getting a confirmed diagnosis, despite the virus isolation being performed at no cost by the University of Kentucky. Performance horses were the most frequently vaccinated, some being vaccinated 4-6 times a year but, despite this, 2- and 3-year-olds were still involved in outbreaks. There were anecdotal reports that breeding farms that delayed their foal vaccinations until after 6 months of age experienced fewer problems than in the past.

**Florida (L. Coffman)**

Laboratories in Florida only average one isolation of influenza virus per year and it was felt there was a real need for a stall-side diagnostic test for attending clinicians to facilitate a rapid diagnosis.

**California (S. Hietala)**

In the fall of 1997, California experienced the first in a series of equine influenza-associated outbreaks since the 1992 isolation of a virus that was identified as being identical to A/eq/Alaska/91 (H3N8). The California Veterinary Diagnostic Laboratory System isolated an influenza virus that caused severe, highly contagious respiratory disease in horses in California through to the spring of 1999. Cross-neutralisation studies indicated that the H3N8 virus appears to have drifted from the prototype Alaska/91 and Kentucky/94 isolates but sequence analysis and genetic typing has not been performed to date. The first cases of severe disease were reported in horses that had recently travelled to a neighbouring state. The outbreak was later reported in private barns, breeding farms and Thoroughbred lay-up facilities in central and southern California and cases continue to be reported in to the summer of 1999. Clinical signs

**TABLE 5: IURD investigations with confirmed influenza virus infections in Ohio, USA**

Month/year	Location	Approximate population size	No. seroconverted	No. Directigen +ve	No. influenza isolated
6/98	Columbus	700	1/1	1/1	0/1
7/98	Akron	120	9/12	6/12	4/12
8/98	Cleveland	1000	6/11	2/11	0/11
8/98	Columbus	20	1/2	0/2	0/2
5/99	Columbus	4	ND	3/4	3/4
5/99	Columbus	60	ND	3/4	3/4

ND = not determined

were typical of influenza, including sudden onset fever, depression and respiratory disease of variable severity. Disease occurred in both vaccinated and non-vaccinated horses of all ages with the most severe disease reported in horses that did not have a history of recent vaccination and although many cases were severe, mortality was low. The infection was initially diagnosed by virus isolation and later by a commercial antigen capture ELISA and seroconversions on paired serum samples. HI titres to Alaska/91 and Kentucky/94 frequently exceeded 1/640 within one to 2 weeks of disease onset.

## CENTRAL AMERICA AND CARIBBEAN

### *Barbados (T. King)*

Barbados has not experienced an outbreak of equine influenza in the past 24 years but it is appreciated that there is a risk of this occurring particularly via imports from outside the region, which occur periodically. Under a tripartite agreement between Barbados, Jamaica and Trinidad and Tobago, which was drawn up in 1996, each respective Racing Authority put in place a common vaccination programme with respect to equine influenza (and equine herpesvirus and tetanus) in racing horses, which has facilitated movement between the 3 jurisdictions. The influenza vaccination programme is administered by the Barbados Turf Club (BTC), which makes vaccination mandatory for racing Thoroughbreds but only voluntary for other horses. The BTC programme requires that, for registration, horses be vaccinated with a first dose at 3 months, a second dose at 4 months and these be followed by booster vaccinations every 6 months during the horses' racing career. Horses entering BTC premises must have evidence of adequate vaccination and must have been vaccinated between 7 and 30 days prior to entry. Failure to maintain a horse's vaccination schedule requires that animals start a primary course again with fines imposed on offending trainers and possible removal of offending horses from premises. All vaccinations must be performed by registered veterinary surgeons and entered on the official form issued by the BTC. There are currently no requirements for the vaccination of breeding stock and this is generally not performed, even in pregnant mares, as this would require a change in the timing of foal vaccination. The

majority of vaccines used are those produced by Fort Dodge but some horses are vaccinated with Prestige and Intervet products.

### *Jamaica (G. Grant)*

Equine influenza was not officially diagnosed in Jamaica prior to December 1989 when a severe outbreak of respiratory disease occurred at the Caymanas racetrack. Initially 200 horses were affected with signs of dullness, lethargy, poor appetite, nasal discharge and raised temperatures. Over the following 5 days some 900 horses were affected with all stables at the racetrack and others outside having cases. The Veterinary Services Division of the Ministry of Agriculture implemented strict quarantine measures and movement restrictions. Laboratory tests confirmed the diagnosis of infection with H3N8 subtype influenza virus. Investigations indicated that the outbreak originated from horses returning via the USA from the Classico International del Caribe race meeting held in Puerto Rico. Following this outbreak a compulsory vaccination programme was initiated. In April 1998 another outbreak of influenza-like illness was reported from the Caymanas racetrack with 124 of 1,200 horses at the racetrack and surrounding area being affected. Limited quarantine and movement restrictions were imposed, and laboratory testing of single blood samples from cases and a review of the vaccination programme were undertaken. No further cases were reported by the end of May 1998. Influenza was suspected but was not confirmed by the conventional methods of seroconversion on paired sera or demonstration of viral antigen by virus isolation or ELISA.

### *Trinidad (T. King on behalf of D. Kangaloo)*

An outbreak of equine influenza occurred in Trinidad in 1979 following the importation of horses from the UK, in which 90% of horses in training at the racetrack were affected and 12 days' racing were cancelled over a 12-week period. In January 1986 another outbreak occurred following the importation of infected horses in which affected contacts had only been vaccinated the previous autumn. In 1994 racetracks were closed for 4 months from 26th February following an H3N8 outbreak. This outbreak resulted from the importation of vaccinated 2-year-olds from the sales in Ocala. The imported horses were sent to a farm from which a local 2-year-old was sent to the

**TABLE 6: Equine influenza outbreaks in Argentina**

Year	Months	Virus	Outbreak type
1976/77	Nov–Mar	A/eq/Argentina/76 (H7N7)	Widespread epizootic
1985/86	Nov–Jan	A/eq/La Plata/85 (H3N8)	Widespread epizootic
1988	Jan	A/eq/La Plata/88 (H3N8)	Outbreak
1989	Jan–Nov	A/eq/La Plata/89 (H3N8)	Outbreak
1993	Feb–Mar	A/eq/La Plata/93 (H3N8)	Racetracks outbreak
1993	Jun		Breeding farm outbreak
1993	Sep		Breeding farm outbreak
1994	Apr–May	A/eq/La Plata/94 (H3N8)	Localised mild outbreak
1994	Sep		Racetracks outbreak
1995	May–Jul	A/eq/La Plata/95 (H3N8)	Localised mild outbreak
1996	Aug		Outbreak
1996	Nov	A/eq/La Plata/96 (H3N8)	Localised mild outbreak
1997	May	A/eq/Argentina/97 (H3N8)	Outbreak
1999	Jan–Feb	A/eq/Argentina/98 (H3N8)	Outbreak

racetrack 7 days later, clinical signs appeared in the horses at the racetrack one week later.

### *Mexico (J. Garcia-Garcia)*

Mexico has diagnostic services provided by one laboratory for an equine population of 4.43 million horses, which are used for sporting, exhibition and farming purposes. In 1997 there were 17 outbreaks of equine influenza diagnosed in Mexico involving 175 cases and including 5 deaths. There have been no reports of influenza during 1998 or so far in 1999 because the only diagnostic laboratory is no longer performing tests. There are 6 equine influenza vaccines available from 3 pharmaceutical companies in Mexico and these are produced as either single or mixed antigen products.

## **SOUTH AMERICA**

### *Argentina (E. Nosetto, M. Barrandeguy)*

The equine industry is important in Argentina and comprises around 2 million animals of several breeds spread throughout the country. Large numbers of horses used for sporting purposes are found around the cities with the remainder used in livestock movement. Prior to 1976 no equine influenza epizootics were recorded in Argentina but a major epizootic caused by an H7N7 virus in October of that year that affected the majority of horses in the country. Infection with this strain of virus has not been recorded since. In November 1985, a second epizootic affecting all regions of the country began. This led to racing and other events being cancelled for almost 2 months and farming was also affected with the estimated losses from

this outbreak being in the region of US\$60 million. An H3N8 subtype virus was isolated from this major epizootic and since then influenza has been reported almost every year with H3N8 viruses being isolated periodically. Vaccination against equine influenza is compulsory in horses that travel frequently within the country with the National Veterinary Service controlling the use of both imported and home produced vaccines. Table 6 shows details of the influenza outbreaks that have occurred in Argentina.

An indirect antigen capture ELISA was useful in the rapid screening and detection of equine influenza virus in nasal swabs. It has been shown that influenza continues to be an important cause of economic loss to the equine industry in Argentina as it is the cause of outbreaks of varying intensity almost every year. Outbreaks continue to occur despite the use of vaccination, with infected horses having little or no evidence of post vaccination HI antibody at the time they are infected, indicating poor vaccine performance. H7N7 subtype viruses continue to be isolated in the 1990s with some evidence of antigenic change in recent isolates. It is suggested that vaccination with reliable vaccines in conjunction with serum surveys to monitor immune status may be a useful tool for controlling equine influenza.

### *Chile (M. Perez)*

Equine influenza affecting yearlings and 2-year-old horses in Santiago and Concepcion, and presumed to be the H3N8 subtype, was first diagnosed in Chile in 1963. In 1977 an H7N7 subtype virus was identified for the first time. Since then there have been periodic outbreaks of disease particularly in the

**TABLE 7: Summary of influenza outbreaks diagnosed in the United Kingdom 1997 to mid-1999**

Month/ year	Area	Diagnostic method	TB/ non-TB	Clinical signs					Vacc'n status	Virus isolated
				Severity	P	ND	C	Other		
5/97	Scotland	ELISA	Non-TB	Mod.	+	+	+		-	-
6/97	Scotland	ELISA and HI s/c	Non-TB	Mod.	+	+	++	Link to May outbreak	-	-
6/97	Wales	ELISA	Non-TB	Mod.	+	+	+		-	-
7/97	Scotland	ELISA	Non-TB	Mod.	+	+	++		-	-
10/97	Lambourn	HI s/c	TB (NH)	Mild			+		+	-
11/97	Lambourn	HI s/c	TB (flat)	Mod.					+	-
11/97	Cumbria	NS1 and HI titre	Non-TB	Mod.	+	+	+		-	-
2/98	Lambourn	HI s/c	TB (NH)	Mild	+/-	+	+/-	Wt loss and poor performance	+	-
2/98	Suffolk	ELISA HI s/c	Non-TB	Severe	++	++	++	Pneumonia	-	+
3/98	Yorkshire	HI s/c	TB (NH)	Mod.	+	+	+			-
3/98	Kent	ELISA	Non-TB	Mod	+	+		<i>S. equi</i> PCR +ve		-
4/98	N'market	ELISA HI s/c	TB (flat)	Mild	+	+	+		+	+
5/98	Scotland	ELISA	Non-TB	Mod.	++			Resp. noise		+
6/98	Wales	NS1 and HI titre	Non-TB	Severe	++	+	+	Depression	-	-
12/98	Wales	HI s/c	TB (P-P)	Severe			++			-
12/98	Lambourn	HI s/c	TB (flat)	Mod.	++	+	+		+	-
1/99	Somerset	HI s/c	Non-TB	Severe	++	++	++	Anorexia	-	-
2/99	Suffolk	ELISA	Non-TB	Severe	++			Dyspnoea	-	-
6/99	N'market	HI s/c	TB (flat)	Mild	+/-	+	+/-	Poor performance	+	-

s/c = seroconversion, P = pyrexia, ND = nasal discharge, C = cough, TB = Thoroughbred, NH = national hunt, P-P = point-to-point, Mod. = moderate

central and northern regions of the country where most of the movement of horses occurs. There were no reports of outbreaks of equine influenza in 1994/1995 but since then there were 3 outbreaks with 10 cases in 1996, 7 outbreaks (303 cases) in 1997 and 14 outbreaks (106 cases) including one equine death in 1998. There have been no reports of disease to date in 1999. Records of authorised vaccine doses show that there are only around 23,000 doses available for the population of around 450,000 horses, which is sufficient to provide approximately 5% vaccine coverage. The majority of vaccine is used when outbreaks are suspected and only certain racing clubs adopt routine vaccination.

## EUROPE

### *United Kingdom (J. R. Newton)*

Details of equine influenza virus outbreaks that have occurred in the United Kingdom between 1997 and mid-1999 are summarised in Table 7. Table 8

summarises outbreaks of influenza that have occurred in vaccinated Thoroughbreds since 1997.

Details of equine influenza vaccines licensed for use in the UK are summarised in Table 9. In the UK, different equine bodies stipulate their own regulations with respect to equine influenza vaccination. These vary considerably between different breeds or disciplines and range from no mandatory vaccination to the Jockey Club Rules, which are the most stringent. The Jockey Club Rules were made mandatory for horses attending British racecourses at the start of the flat racing season in March 1981. They require that horses have a properly certified vaccination record that includes 2 injections given 21–92 days apart, which is then followed by a first booster injection given between 150 and 215 days ('6-month' booster) after the second injection of the primary course. Subsequent booster injections are given at intervals of not more than 12 months. No vaccinations are to be given within 7 days of entry onto a racecourse. Influenza vaccination

**TABLE 8: Influenza in vaccinated Thoroughbreds in the UK since 1997**

Year	Month	TB (type)	Severity	Last vaccination
1997	Oct	National Hunt	Mild	Not known
1997	Nov	Flat (yearling)	Moderate	5 months after 1 <sup>st</sup> course (2 doses)
1998	Feb	National Hunt	Mild	6–7 months after last booster
1998	Apr	Flat (2- and 3-year-olds)	Mild	5 months after last booster
1998	Dec	Flat (3-year-olds)	Moderate	12 months after last booster
1999	Jun	Flat (2- and 3-year-olds)	Moderate	6 months after last booster (some 1 <sup>st</sup> )

**TABLE 9: Datasheet information for UK licensed influenza vaccines in 1999**

Vaccine name	Vaccine company	Type/ adjuvant	Viral strains interval	Primary course interval	First booster interval	Subsequent booster interval
Duvaxyn IE Plus	Fort-Dodge	Carbomer adjuvant	H7N7: Prague/56 H3N8: Miami/63 & Suffolk/89	4–6 wks	6 mnths	12 mnths
Equip	Schering-Plough	ISCOM	H7N7: Newmarket/77 H3N8: Brentwood/79 & Borlange/91	6 wks	5 mnths	12–15 mnths
Prevac Pro	Hoechst Roussel Vet	Aluminium hydroxide adjuvant	H7N7: Prague/56 H3N8: Miami/63 & Newmarket/2/93	4–6 wks	5–7 mnths	12 mnths

requirements for the Federation Equestre Internationale (FEI) are similar to the Jockey Club Rules except there is no requirement for the first '6-month' booster injection.

### ***Ireland (A. Cullinane)***

Mandatory equine influenza vaccination was introduced in Ireland in 1980 and between then and 1989 there were no outbreaks of the disease in the country. There was a major epizootic in 1989 however which started in December in horses attending the Royal Dublin Horse Show and since then there have been sporadic outbreaks. The Irish Turf Club rules for vaccination are in line with those outlined for the Jockey Club in the UK and have been largely effective in producing control nationally in that no racing has been cancelled since their introduction. Recommendations from the Irish Equine Centre are for booster vaccinations to be administered every 6 months. A variety of influenza vaccines are available, in addition to those available in the UK there is Fluvac from Fort Dodge and NobieQuenza from Intervet. Diagnostic techniques include virus isolation in eggs and the Directigen ELISA that has been found to be more sensitive than virus isolation. Serological testing

routinely uses the HI test but SRH tests are adopted in vaccine trials. The Kildare/89 strain was characterised as a European-like virus, whereas Kildare/92 was found to be American-like and Kildare/95 has not been characterised. Sporadic outbreaks that occurred in 1997 and 1998 and affected only a few premises have illustrated the increasing difficulty in diagnosing influenza in vaccinated horses and has led to some dissatisfaction with vaccines amongst some trainers, who expect complete protection.

### ***Sweden (B. Klingeborn)***

Twenty-two outbreaks of influenza were recorded in Sweden in 1998 and 10 so far in 1999. Vaccination coverage is poorer in northern Sweden, ranging from 10% to 30%, compared with the rest of the country where it is between 40% and 70%. The compulsory vaccination policy that was adopted in 1979 was made only voluntary in March 1993. The outbreak that persisted from November 1998 to January 1999 was the largest since 1992–1993. This outbreak affected horses at 11 racetracks in a 7-week period whereas the outbreak in 1992–1993 affected 22 racetracks and lasted 29 weeks. Disease incidence in this most recent outbreak varied from

**TABLE 10: Age-specific geometric mean HI titres against H3N8 vaccine viral strains for racehorses at 2 training centres in Japan, 6 months after vaccination**

Age group	Ritto Training Centre		Miho Training Centre	
	Kentucky/81	La Plata/93	Kentucky/81	La Plata/93
2-year-olds	60	67	87	75
3-year-olds	97	52	89	62
4-year-olds	80	84	111	72

20% to 80% with 2% to 25% of horses becoming very sick. Overall 11% of stables had very sick horses compared with only 4.5% in the 1992–1993 outbreak. An American-like H3N8 virus was found to be the cause of the outbreak. In the last 5 years the policy has changed from isolation of the whole racetrack to isolation of only the affected stables. Five days after the last case has recovered isolation of the affected stables is lifted. In this outbreak half of the affected racetracks did not need to isolate all the stables on the track and when this did happen not all stables were isolated at the same time. Isolation of influenza viruses in eggs has proved very difficult with no isolations being made in eggs since 1996 despite 300 attempts. Sequencing of the HA gene of 20 isolates identified by a direct nested RT/PCR show that 19 of them belong to the American lineage.

## MIDDLE AND FAR EAST

### *United Arab Emirates (R. Wernery)*

The last outbreak of equine influenza occurred in the United Arab Emirates in December 1995 following the importation of horses from America to a polo pony club and was relatively minor and contained. Since then there have been stringent quarantine testing and vaccination requirements for horses entering the country and these to date appear to have been effective in preventing the introduction of equine influenza. In 1996, a serosurvey conducted among Thoroughbred and pleasure horses showed that 43% of horses were considered adequately immunised against H7N7 influenza and 22% were immune to H3N8 viruses. A survey of samples from 500 camels showed no antibodies against either H7N7 or H3N8 viruses.

### *Japan (T. Matsumura)*

The last equine influenza epizootic was caused by the A/eq/Tokyo/71 (H3N8) strain and occurred in Japan between 4th December 1971 and 11th

January 1972, affecting 6,800 horses.

The influenza vaccination policy of the Japanese Racing Authority (JRA) prior to June 1998 comprised a primary vaccination of 2 injections as yearlings and this was followed by annual booster vaccinations. Since the middle of 1998, the official JRA policy has stipulated that booster vaccinations be administered every 6 months in May and November. Inactivated vaccines used in Japan since September 1996 have included A/eq/Newmarket/77 (H7N7), A/eq/Kentucky/81 (H3N8) and A/eq/La Plata/93 (H3N8) antigens. Table 10 shows the age-specific geometric mean HI titres against H3N8 vaccine strains in blood samples taken in November 1998, 6 months after vaccination, from racehorses at 2 training centres.

### *Philippines (J. A. Mumford)*

A major outbreak involving 1,800 horses occurred on 2 racetracks and the polo club in Manilla in 1997. Approximately 90% of horses were affected. The infection was diagnosed by detection of antigen using the nucleoprotein antigen capture ELISA and detection of NS1 antibody in convalescent sera in samples sent to the AHT. The source of infection was presumed to be horses recently imported from the USA and the H3N8 virus isolated was typed as belonging to the American lineage.

## DISCUSSION

### *J. A. Mumford*

The information presented indicates that more effort is being made internationally for surveillance and improved diagnosis.

### *D. P. Lunn*

Does the situation regarding equine influenza status differ between America and the UK? Previously, influenza was considered to be endemic in America whereas the UK was considered to be fairly influenza-free, has this

situation changed?

**J. A. Mumford**

Between 1979 and 1989, the UK and Ireland were mostly free of influenza. However, since 1989, influenza has been endemic in the UK.

**P. Gibbs**

Is vaccination perpetuating endemicity? How does the situation compare between vaccinated and native horses on islands (eg Barbados)?

**T. King**

On Barbados, horse population numbers are too low to support endemic influenza (approximately 1,500 horses); therefore it is easier to control the disease.

**J. A. Mumford**

One problem affecting the ability to conduct surveillance programmes is that there is often no perceived benefit amongst practitioners and owners in collecting data on virus outbreaks due to questions concerning vaccine efficacy.

There is a need to improve collation and dissemination of information relating to the global surveillance effort. This may be achieved by, for example, better and more rapid communication via e-mail or via a web site.

**Question**

Would a web site be acceptable for presenting data from the International Collating Centre as participants pay a subscription and therefore want privileged access to information submitted?

**J. A. Mumford**

Confidentiality of information must be taken into account when establishing a web site, but this should be possible by having restricted access to potentially sensitive information.

In the UK, a reporting system is encouraged via 'yellow forms' for notification of equine influenza vaccine failure. Is there a similar system in the USA?

**T. Chambers**

There is no formal system in the US.

**R. Miller**

Systems are available within the USDA; the Biologicals Programme provides advice upon reporting of vaccine failure to veterinary practitioners and also to the American Pharmacopoeia. A mandatory system for farmers to log complaints is being developed, but details are not available yet.

**D. P. Lunn**

In answer to whether vets understand the value of reporting vaccine failure when influenza vaccines are not perceived to be very effective in the USA, it is stated in a standard Veterinary textbook (Smith) that there is 'no point in testing' to confirm suspected influenza.

**J. A. Mumford**

To be effective, it is important to establish a network of scientists and informed practitioners. Often there is a problem in obtaining paired serum samples due to the extra cost and inconvenience for the horse owner. Virus isolation is often unsatisfactory from swab samples and there is a need to communicate the availability of improved sampling and testing methods.

**D. Wilson**

There is general dissatisfaction amongst practitioners with the requirement for, and effect of, surveillance. It is important to disseminate new test methods so people know the clinical benefits of obtaining early, reliable diagnosis.

**J. A. Mumford**

There is clearly a need to raise the expectations of both vaccines and diagnostic tests.

**A. Glaser**

It is useful to test in the field using the Directigen test to identify virus positive animals and then collect samples. In order to extend virus isolation outside New York State, it would be necessary to obtain external funding support.

**P. Gibbs**

As part of a student project, questionnaires associated with equine influenza were distributed to 300 veterinarians in Florida. There was a general apathy for returning responses and no interest in obtaining diagnoses to confirm suspected influenza outbreaks. It was clear that the practitioners were not expecting much of vaccines.

**R. Miller**

Although some vaccines do not work well, others incorporating newer strains are more effective and it should not be concluded from this meeting that American vaccines are no good.

**D. G. Powell**

When laboratories get swab samples that are positive by the Directigen test, there is a high probability of being able to isolate virus and carry out further testing.

**R. Miller**

The importance of diagnostic kits is emphasised within the USDA. Is there a recognised 'gold standard' for diagnosis of equine influenza?

**T. Chambers**

A 'gold standard' is not yet established. The Animal Health Trust's antigen capture ELISA is more sensitive than other tests, but not yet commercially available. For virus isolation, it should be noted that for recent isolates it may be better to use MDCK cells than eggs. It is important to get the message across to diagnostic laboratories that tests have improved.

**D. G. Powell**

Currently there is no diagnosis of influenza in Texas. Recently the racing industry in Texas has grown and with the large number of horses in Texas there is likely to be increasing interest in disease surveillance.

**R. Holland**

Veterinarians may be concerned about confidentiality. When encouraging them to participate in reporting of suspected influenza outbreaks it is important that these concerns are allayed. If the idea of a quick, reliable test for influenza is emphasised, veterinarians are more likely to co-operate in provision of samples. It should be noted that veterinarians dislike using large swabs for obtaining samples.

**D. P. Lunn**

We use smaller (6 inch) swabs, which are in fact human vaginal swabs, and have no difficulty in obtaining isolates during experimental infection studies.

**H. Townsend**

During experimental infections, Dacron swabs have been reliable for isolation up to Days 7 or 8 post infection. These are likely to be more efficient than cotton swabs since they have been designed specifically to hold and then release virus and hence smaller swabs are effective.

**P. Morley**

Proctoscopic swabs (designed for humans) work very well in horses.

**J. Traub-Dargatz**

Six-inch swabs work fine for virus isolation and the Directigen test, with these swabs there have been no objections from vets and trainers as they are quicker to use and cause less irritation to horses.

**D. Wilson**

The nature of swabs is the cause of much dissatisfaction amongst vets. It would be useful to standardise swabs and sampling procedures that work effectively and will receive good acceptance from vets.

# SESSION 2:

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## Antigenic and genetic characterisation of viruses

*Chairman: T. Chambers*

## INTRODUCTION

Only a relatively few laboratories are undertaking detailed characterisation and comparison of equine influenza isolates. Genetic analysis is based on nucleotide sequence of the HA and other genes. Antigenic analysis is based on serological tests using ferret and horse sera and monoclonal antibodies. The dichotomy of European lineage

and American lineage HA of H3N8 subtype viruses was first reported at the previous meeting in this series in 1995, after the UK and American OIE reference laboratories pooled their results to get a 'worldwide' picture. The divergence of H3N8 viruses is the framework within which evolution of H3N8 viruses is currently described.

## GENETIC ANALYSIS BASED ON NUCLEOTIDE SEQUENCE OF THE HA AND OTHER GENES

### A. Lai

Phylogenetic clusters of isolates were identifiable when genetic analysis of isolates from distinct locations in the Americas was performed, namely Kentucky, Florida and Argentina (Fig 7). The rates of evolution and also the 'hot spots' of amino acid substitution differed among these clusters. This reinforces our dogma that regular and comprehensive international surveillance is necessary to ensure that our understanding of H3N8 virus evolution is based on truly representative viruses.

### N. Davis-Poynter

The latest isolates to be characterised at the Animal Health Trust include isolates from Dubai (1995), the Philippines (1997) and Czechoslovakia (1997) as well as the UK (1998). These were all of the American lineage as seen by the greater amino acid similarity to Newmarket/1/93 than to Newmarket/2/93 (Table 11).

It was relatively difficult to isolate these viruses in eggs, compared to MDCK cells. Two isolates that could not be isolated in eggs (Moulton/98 and Edinburgh/98) possessed lysine instead of glutamine at position 190, similar to the report of Dr Lai. This raises the possibility that fewer viruses may be isolated due to their inability to grow in eggs.

### A. Hay

During 1998 and 1999, 15 equine influenza H3N8 viruses isolated in 1989 were analysed at the WHO Influenza Centre, Mill Hill, London. These included one isolate from Austria, and 14 from Italy, isolated from horses imported from Poland. The Italian isolates were closely related

antigenically to Sussex/89 and Newmarket/2/93 although sequence analysis of the HA revealed that they possessed 3 amino acid substitutions from the majority of earlier isolates. Results of comparative sequencing of neuraminidase genes, reported for

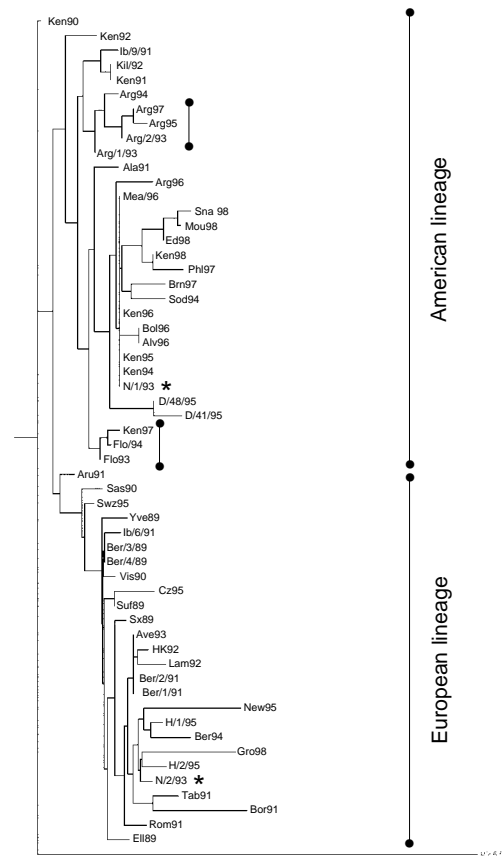


Fig 7: Phylogenetic tree derived from HA1 amino acid sequences of H3N8 equine influenza strains.

**TABLE 11: Amino acid changes in putative antigenic sites and distinction between strains of American (Newmarket/1/93) and European (Newmarket/2/93) lineages**

Virus	Antigenic site/amino acid position														
	E				A			B			D				
	48	57	58	78	112	135	144	163	189	190	207	213	214	222	228
<b>Reference strains</b>															
Mia/63	T	R	V	V	V	G	A	T	N	E	K	I	I	W	G
N/1/93	I	.	.	.	.	R	.	.	Q	Q	.	.	.	.	.
N/2/93	I	.	.	D	.	.	.	I	K	.	E	V	.	.	.
D/41/95	I	.	.	.	.	R	.	.	Q	K	.	.	.	R	.
D/48/95	I	.	.	.	.	R	.	.	Q	K	.	.	.	R	.
Phl/2/97	V	K	.	.	.	R	T	.	Q	Q	.	.	V	.	.
Brn/97	I	.	.	.	I	R	.	.	Q	Q	.	.	.	.	.
Ed/98	I	K	.	.	.	R	.	.	Q	K	.	.	.	.	.
Mou/98	I	K	I	.	.	R	.	.	Q	K	.	.	.	.	.
Sna/98	I	K	I	.	.	R	.	.	Q	X	.	.	.	.	S

**TABLE 12: Amino acid changes in HA1 of Argentinian isolates**

Virus	Amino acid position (antigenic site)												
	70	78 (E)	126	135 (A)	138 (A)	157	163 (B)	166 (B)	172 (D)	189 (B)	190 (B)	207 (D)	213 (D)
Mia/63	M	V	T	G	A	S	T	V	K	N	E	K	I
N/1/93	.	.	.	R	.	.	.	.	Q	Q	.	.	.
N/2/93	.	D	.	.	.	.	I	.	.	K	.	E	V
LP/93	.	.	.	.	.	.	.	.	.	.	.	.	.
LP/95	.	.	I	.	S	Y	.	.	E	.	.	.	.
LP/96	I	.	I	.	S	.	.	M	E	.	.	.	.

the first time at these meetings, mirrored the Eurasian/American divergence previously reported for the HA.

### **B. Klingeborn**

Of 20 isolates from 1995/96 in Sweden, 19 were of the American lineage. No viruses had been isolated since 1996, apparently due to the inability of recent strains to grow in eggs. Those isolates that grew in eggs had glutamine 190, whereas those that were isolated in MDCK cells had lysine at position 190. Passage of the cell-isolated viruses in eggs triggered a lysine to glutamine substitution at that position. This raises the issue of host cell selection of equine influenza viruses and its relevance to vaccines. The situation has parallels with the

experience of the WHO with human H3 influenza. Nonetheless, Dr Lai reported that direct RT-PCR sequencing from nasal swab virus without prior passage sometimes still yields the glutamine 190 genotype (egg-like).

### **M. Pecoraro**

The most recently characterised Argentinian isolates were La Plata/95 (LP/95) and La Plata/96 (LP/96). These were related most closely to La Plata/93 and Kentucky/94 by sequence analysis of the HA (98% and 97% amino acid homology, respectively). Amino acid changes among the Argentinian isolates are given in Table 12. Phylogenetic analysis revealed that LP/93, LP/96 and LP/96 formed a unique branch of the American lineage.

## ANTIGENIC ANALYSIS BASED ON SEROLOGICAL TESTS USING FERRET AND HORSE SERA AND MONOCLONAL ANTIBODIES

### A. Hay

It was concluded that, in addition to the increase in 'American-like' viruses in certain European countries, European type viruses continue to circulate without significant changes in antigenicity of the majority of isolates studied. One Italian virus had reduced HI titres with ferret sera to Sussex/89, Newmarket/1/93, Newmarket/2/93 and several monoclonal antibodies even though its HA sequence was similar to HA sequences of contemporary isolates. It did, however, have a deletion of 13 NA residues (54–66) in the stalk

region. This raises the possibility that changes in NA could affect antigenicity in the HI test.

### P. Yates

Ferret sera to Newmarket/1/93 or Newmarket/2/93 can usually distinguish between Eurasian and American isolates, but with some anomalies. In particular, the anti-Newmarket/2/93 serum is fairly specific for Eurasian strains. Recent American-lineage isolates all reacted well with the anti-Newmarket/1/93 serum (Table 13). A few 'high avidity' isolates, such as Newmarket/95, are more difficult to characterise by antigenic

**TABLE 13: Antigenic analysis of 1997/1998 isolates with ferret sera**

Virus	Ferret sera raised to									MAB 7.4A1 /2
	N1/93	N2/93	C/3/95	H/1/95	S/2/95	D48/95	B/97	P/2/97	Ed/98	
Brno/97	≥1024	16	256	32	256	256	≥1024	≥1024	≥1024	12288
Phil/2/97	512	16	256	32	384	256	≥1024	≥1024	512	4096
Snailwell/1/98	768	256	512	256	512	256	≥1024	≥1024	≥1024	4096
Moulton/1/98	512	48	256	64	512	256	≥1024	≥1024	512	8192
Edinburgh/1/98	256	16	512	64	256	256	≥1024	≥1024	<b>512</b>	4096
<b>Reference strains</b>										
N/1/93	<b>256</b>	<8	32	32	128	32	384	≥1024	256	4096
N/2/93	64	<b>128</b>	64	768	<8	<8	<8	8	<8	<8

Homologous titres are in bold

**TABLE 14: HI titres of sera from vaccinated 3- to 5-year-old Argentinian horses**

HI titre	Frequency of HI titres against different strains*						
	Tk/71	Ken/81	Suf/89	Ken/94	LP/93	LP/95	LP/96
4	10	9	45	10	3	9	9
8	15	10	33	18	6	12	15
16	19	24	12	35	13	36	31
32	32	27	5	27	29	29	33
64	15	18	-	5	34	9	7
128	4	7	-	-	8	-	-
256	-	-	-	-	2	-	-
512	-	-	-	-	-	-	-
Total	95	95	95	95	95	95	95

\*Tk/71 = Tokyo/71; Ken/81 = Kentucky/81; Suf/89 = Suffolk/89; LP/93 = La Plata/93; LP/95 = La Plata/95; LP/96 = La Plata/96

analysis because they cross-react well with most sera.

***T. Chambers***

The highlight of antigenic characterisation of recent US isolates is that, based on very preliminary data, Kentucky/99 might represent a significant antigenic variant and needs to be examined further.

***M. Pecoraro***

The reactivity patterns of the Argentinian isolates with post infection ferret antisera were distinct from the reactivity pattern of the European isolate Suffolk/89. Although ferret antiserum to Suffolk/89 cross-reacted fairly well with the American isolates, there was little reactivity of sera raised against American isolates with Suffolk/89. Of a panel of 95 serum samples from 3- to 5-year-old vaccinated Argentinian horses, only 5 had an HI titre of 1/32 against Suffolk/89 compared with 27–33 with an HI titre of 1/32 against Kentucky/94 and the recent Argentinian isolates (Table 14). Poor cross-reactivity of antibodies stimulated by current

equine influenza vaccines in Argentina with European strains such as Suffolk/89, potentially leaves Argentinian horses susceptible to outbreaks from a European strain. The conclusion from this is that Argentinian vaccines need to be updated with European as well as American strains.

**DISCUSSION**

There has not been a confirmed isolation of an H7N7 subtype virus for over 20 years. The few reports of seroconversion to H7N7 virus could potentially be accounted for by cross-reaction with H3N8 virus.

The possibility of omitting H7N7 subtype representatives from vaccines was discussed, and there seemed to be a consensus that inclusion of an H7N7 representative in the vaccines offered lacked credibility as an epidemiologically relevant strain. Therefore, the OIE recommendations for vaccine strain composition should no longer include the H7N7 component. However, this does not absolve those involved in diagnosis and surveillance from continuing to watch for H7N7 isolates.



# SESSION 3:

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## Vaccine strain selection scheme

*Chairman: N. Davis-Poynter*

*Rapporteurs: A. Hay and B. Klingeborn*

## REPORT ON ACTIVITIES OF THE EQUINE INFLUENZA EXPERT SURVEILLANCE PANEL

J. A. Mumford

At the 1995 consultative meeting of WHO/OIE experts, it was agreed that there is a requirement for a formal reporting system for equine influenza variation and for advice to vaccine manufacturers for updating strains. This led to establishment of an expert surveillance panel. The panel meets annually to review the epidemiology of outbreaks, genetic and antigenic variation of currently circulating isolates, and formulation and performance of available vaccines. On the basis of these meetings, recommendations are made for the selection of strains for incorporation into vaccines and reported each year in the OIE bulletin. The recommendations since 1995 have been to

remove 'ancient' isolates (eg Miami/63) and introduce recent isolates representative of current prevalent strains, with at least one American-like and one European-like strain. It is a requirement of this surveillance programme that information, virus strains and other reagents be exchanged freely between panel members.

In 1998, 6 American and 22 European isolates were reported. Genetic and antigenic analysis indicated an increased proportion of American-like viruses isolated in Europe. Unfortunately, because there was little information available concerning specific outbreaks, and in particular vaccination histories, it was difficult to determine whether vaccine induced immunity was breaking down.

## REVIEW OF CRITERIA USED FOR VACCINE STRAIN SELECTION

J. A. Mumford

The principal considerations of the equine influenza surveillance system are to identify strains currently circulating in the field, to determine the antigenic relatedness between current strains and to identify whether there is any significant antigenic difference from current vaccine strains.

When the equine influenza surveillance and vaccine strain selection programme was first established, the criteria used for selection of human influenza vaccine strains were adopted. As for human influenza, antigenic analysis of equine influenza virus isolates is performed using post infection ferret sera in HI assays, and these data are supported by sequencing of the HA1 gene. For human influenza, the ability of sera from vaccinated volunteers to discriminate between isolates is also examined. Further, for human influenza, observation of infection in individuals known to have received potent vaccines is taken as evidence for the need to update vaccine strains.

Following review of information submitted to the Expert Surveillance Panel in the 4 years since its establishment, it has become apparent that relatively little reliable field information on

vaccine breakdown is available. There is no infrastructure in place to allow evidence of vaccine breakdown to be used as a primary criterion for vaccine strain selection. Serosurveys, in which HI tests are performed on high titre sera from repeatedly vaccinated horses revealed that such sera are highly cross-reactive and are therefore unlikely to be useful in identifying subtle antigenic variants. However, regular serosurveys in which the ability of antibodies in field sera to inhibit the most recent field strains may be useful in identifying major antigenic differences. Genetic analysis has been extremely useful for distinguishing between isolates of the American and European lineages and can enable identification of the source of viruses introduced into new areas via the international movement of horses. However, it is not yet possible to predict the phenotypic behaviour of a virus on the basis of its genetic make-up. Post infection ferret sera used in HI tests have been shown to discriminate variants of equine influenza strains and can be used to identify viruses from the American and European lineages. Cross-protection studies in

horses enable the influence of antigenic variation on vaccine efficacy to be assessed directly. Parallel studies in hamsters will demonstrate whether these would provide a suitable small animal model.

In summary, it is proposed that equine influenza isolates will initially be screened for antigenic variation by HI tests with post infection ferret sera. If antigenic differences of at least 4-fold

from vaccine strains is seen in HI tests with ferret sera, with supporting sequence data, cross-protection studies in hamsters could be performed. If these studies suggest that antigenic drift is having a detrimental effect on vaccine efficacy, cross-protection studies in ponies will also be considered. The rationale for adopting this approach to vaccine strain selection is discussed in more detail below.

## COMPARISON OF SERA FROM DIFFERENT SPECIES FOR ANTIGENIC ANALYSIS

P. Yates

Reciprocal HI tests were performed using sera raised against a panel of equine influenza viruses in guinea pigs, ferrets and hamsters. R-values, a measure of antigenic relatedness between pairs of viruses, were calculated according to the formula  $R = \sqrt{(r1 \times r2)} \times 100$ , where r1 = heterologous titre of virus 1/homologous titre of Virus 2 and r2 =

heterologous titre of Virus 2/homologous titre of virus 1. An R-value of >50% indicates that isolates in a pair are antigenically related, whereas an R-value of <50% indicates that the isolates are antigenically different.

Comparing sera from different species, guinea pig sera tended to be too strain-specific, whereas

**TABLE 15: R-values obtained from HI tests with sera raised in different species**

	Fon/79	Ken/81	Suf/89	Aru/91	N/1/93	N/2/93
<b>a. Equine sera</b>						
Mia/63	<b>32</b>	<b>44</b>	<b>14</b>	<b>13</b>	<b>24</b>	<b>11</b>
Fon/79		73	<b>47</b>	81	89	<b>13</b>
Ken/81			<b>31</b>	<b>25</b>	<b>35</b>	<b>12</b>
Suf/89				<b>42</b>	<b>47</b>	<b>27</b>
Aru/91					99	52
N/1/93						<b>18</b>
<b>b. Guinea pig sera</b>						
Mia/63	<b>39</b>	<b>27</b>	<b>13</b>	<b>7</b>	<b>10</b>	<b>7</b>
Fon/79		<b>21</b>	<b>23</b>	<b>9</b>	<b>28</b>	<b>18</b>
Ken/81			<b>11</b>	<b>15</b>	<b>33</b>	<b>38</b>
Suf/89				<b>16</b>	<b>30</b>	<b>32</b>
Aru/91					<b>8</b>	<b>24</b>
N/1/93						<b>38</b>
<b>c. Hamster sera</b>						
Mia/63	<b>15</b>	<b>9</b>	<b>3</b>	<b>4</b>	54	<b>42</b>
Fon/79		<b>26</b>	<b>26</b>	<b>4</b>	<b>33</b>	<b>42</b>
Ken/81			<b>2</b>	<b>5</b>	<b>9</b>	<b>38</b>
Suf/89				56	<b>4</b>	91
Aru/91					<b>35</b>	88
N/1/93						<b>16</b>
<b>d. Ferret sera</b>						
Mia/63	<b>21</b>	<b>23</b>	<b>27</b>	<b>11</b>	<b>7</b>	<b>14</b>
Fon/79		92	<b>40</b>	51	<b>33</b>	<b>14</b>
Ken/81			<b>19</b>	<b>29</b>	80	19
Suf/89				92	<b>31</b>	76
Aru/91					<b>34</b>	64
N/1/93						<b>20</b>

R-values < 50% in bold

ferret and hamster sera displayed similar specificity, largely in line with corresponding results from equine sera, although with certain anomalies (Table 15). Some isolates display unusually high avidity, with the result that it is difficult to determine antigenic relatedness (eg to American-like or European-like lineages) despite having clear genetic relatedness to one or other lineage. Equine

sera tend to be more cross-reactive although this does not necessarily correspond to cross-protection. Ferret and hamster sera are therefore both suitable tools: a 2-way differentiation indicates major antigenic variation, one way differentiation may indicate one way protection following vaccination (eg American-like versus European-like, discussed further below).

## THE USE OF POST VACCINATION FIELD SERA TO DIFFERENTIATE BETWEEN VIRUS STRAINS

P. Yates

A regular serosurvey has been performed in which equine field sera, often from animals that have received multiple vaccinations, with consequent loss in specificity, are tested in HI assays against recent equine influenza isolates. In order to maximise strain differentiation, virus was used without Tween-80/ether-treatment. In general, no significant differentiation was observed between a panel of isolates, with the exception of a virus isolated in Czechoslovakia in 1995 (Czech/95), against which the sera consistently gave a low geometric mean titre (Table 16). It was suggested that the general lack of differentiation between isolates by field sera meant that results have been uninformative, and the value of continuing with the serosurvey as part of the surveillance programme was questioned.

**TABLE 16: Summary of serosurveys conducted between January and December 1998 and in January and June of 1999**

Antigen	Geometric mean HI titres		
	1998	Jan 99	June 99
Miami/63	16	11	57
Kentucky/81	12	6	40
Suffolk/89	17	10	60
Newmarket/1/93	16	12	65
Newmarket/2/93	19	11	73
Berlin/94	22	nd	nd
Newmarket/1/95	14	10	61
Holland/1/95	14	9	53
Switzerland/2/95	29	30	77
Czechoslovakia/95	9	6	38
Fold difference (Suf/89:Czech/95)	1.9	1.67	1.58
No. sera tested	412	36	36

## SIGNIFICANCE OF ANTIGENIC VARIATION FOR VACCINE EFFICACY - STUDIES IN HORSES

J. A. Mumford

Experimental vaccination/challenge studies in horses are difficult and costly to undertake, but provide the most relevant data when investigating the impact of strain variation upon vaccine efficacy. Four such studies have been carried out at the Animal Health Trust to date.

A vaccination study was performed with isolates pre-dating the division between American-like and European-like lineages and challenging with a later European-like virus (Suffolk/89).

General conclusions from this study are that the older the vaccine strain, the worse protection (measured by virus excretion) afforded (Table 17). In this regard, Miami/63 was found to be particularly ineffective as a vaccine.

All vaccine strains were effective, however, at reducing pyrexia. When extrapolating to the field, this may result in vaccinated animals being asymptomatic but still excreting virus. SRH antibody levels estimated as providing protection

**TABLE 17: SRH antibody and virus excretion from ponies challenged with A/eq/Sussex/89 (H3N8)**

Group	Mean pre-challenge SRH (mm <sup>2</sup> )	% ponies excreting virus	Mean duration of virus excretion (days)
Unvaccinated control	0	100	5.1
Miami/63	144	90	3.6*
Fontainebleau/79	56	90	3.3*
Kentucky/81	70	80	2.5**
Suffolk/89	75	56	1.6***

\*P<0.05, \*\*P<0.01, \*\*\*P<0.0001

against virus excretion for the homologous vaccine group were 140 mm<sup>2</sup> whereas, for heterologous vaccine groups, the SRH value affording protection was 200 mm<sup>2</sup> although, unlike the homologous group, there was no clear threshold for protection.

Two studies were performed in which groups of ponies were vaccinated with European-like (Newmarket/2/93) or American-like (Arundel/91) in one study, Newmarket/1/93 in a second study) strains followed by challenge with a European-like strain (Newmarket/2/93). Interpretation of the data was difficult due to differences in the relative levels of pre-challenge SRH antibody in homologous and

heterologous groups. In the first study, the Newmarket/2/93 vaccine provided better protection than the Arundel/91 (heterologous) vaccine, but SRH values measured using the challenge virus were lower for the Arundel/91 vaccinated group than the Newmarket/2/93 vaccinated group (Table 18). However, pre-challenge SRH antibody levels were similar for both vaccine groups when measured against their respective vaccine strains. In the second study, there was no significant difference in protection between the homologous and heterologous vaccines but, in this case, SRH values measured using the challenge virus were significantly higher for the Newmarket/1/93 (heterologous) vaccinated group than the homologous vaccinated group (Table 18). Finally a study was performed in which groups of ponies were vaccinated with European-like (Newmarket/2/93) or American-like (Newmarket/1/93) strains followed by challenge with American-like (Newmarket/1/93) strain. This experiment is more representative of the current situation in the field as current European vaccines include an updated European-like strain but not an updated American-like strain and infection of vaccinated horses by American-like strains have been observed in the field. In this study, the SRH

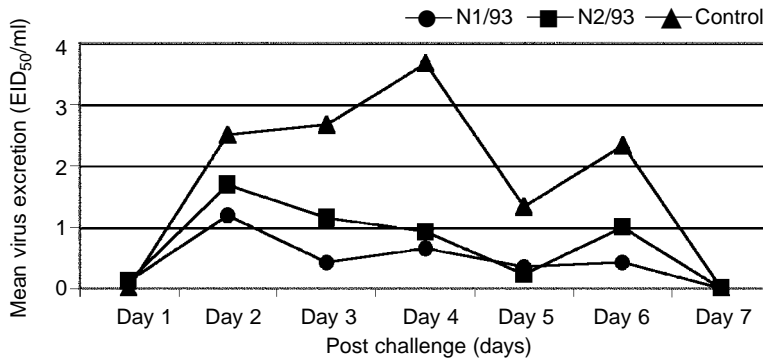


Fig 8: Virus excretion following challenge with Newmarket/1/93 virus.

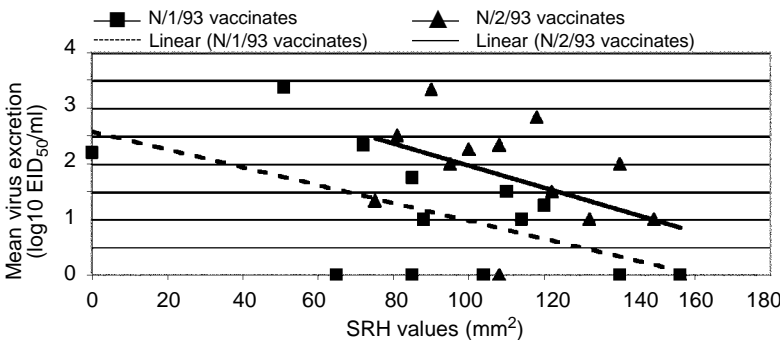


Fig 9: Correlation of amount of virus excreted with pre-challenge SRH antibody values.

**TABLE 18: Summary of pre-challenge SRH antibody and virus excretion following challenge with Newmarket/2/93**

Study No.	Vaccine group	Mean pre-challenge SRH antibody to Newmarket/2/93 (mm <sup>2</sup> )	Virus excretion		
			Mean duration (days)	% ponies excreting virus	Amount (mean EID <sub>50</sub> /ml)
1	Control	0	5.7	100	2.07
	Arundel/91	120	1.3	67	0.41
	Newmarket/2/93	182	0.3	25	0.12
2	Control	0	5	100	1.40
	Newmarket/1/93	119	1.1	40	0.39
	Newmarket/2/93	88	2.2	80	0.5

**TABLE 19: Summary of equine cross-protection challenge studies**

Study	Vaccine strains	Challenge strain	SRH Ab (mm <sup>2</sup> )	Vaccine efficacy (suppression of virus excretion)
1	Miami/63	Sussex/89	144	Efficacy correlated with antigenic relatedness despite high levels of pre-challenge antibody in Miami/63 group
	Fontainebleau/79		56	
	Kentucky/81		70	
	Suffolk/89		75	
2	Arundel/91	N/2/93	88	N/2/93 vaccine more effective but could be related to antibody level
	Newmarket/2/93		119	
3	Newmarket/1/93	N/2/93	119	No significant difference. Higher antibody in N/1/93 group may have compensated for strain variation
	Newmarket/2/93		88	
4	Newmarket/1/93	N/1/93	104	Homologous vaccine more effective despite lower antibody level.
	Newmarket/2/93		113	

titres (measured against the challenge virus) for the heterologous group were only slightly higher than for the homologous group, the preferred situation when assessing the relative protective efficacy against challenge. Both vaccines were effective at reducing clinical signs of disease. When measuring virus excretion, however, the homologous vaccine was significantly more effective than the heterologous vaccine (Fig 8). When correlating individual responses to protection, significantly higher SRH levels were required for the heterologous vaccine group, for protection against both the duration and titre of virus excretion, than for the homologous group (Fig 9).

In conclusion, equine challenge studies (summarised in Table 19) have clearly demonstrated the potential for recent isolates to escape protection afforded by vaccination with current vaccine strains. When considering protection, it is important to distinguish between protection against clinical signs and protection against excretion. Experimentally, vaccine strains

affording low protection against virus excretion are still effective at reducing clinical signs, which is undesirable due to the likely result of 'silent' shedders in the field reducing the effectiveness of outbreak control measures. The most recent studies have compared American-like and European-like strains for cross protection. These strains demonstrate one-way antigenic differentiation, ie ferret sera raised against Newmarket/1/93 cross react by HI with Newmarket/2/93, but sera against Newmarket/2/93 do not similarly cross react with Newmarket/1/93. Even more significant differentiation is expected if similar studies are undertaken comparing virus strains displaying 2-way antigenic differentiation.

## REFERENCES

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# STUDIES IN HAMSTERS

P. Yates

In view of the difficulties and costs involved in equine challenge studies, an alternative small animal model is being investigated. Serological studies indicate that sera from hamsters give similar results to ferrets regarding antigenic differentiation between strains, which are broadly in line with (albeit more discriminatory than) the differentiation observed with equine sera. Hamsters can be infected with non-adapted equine influenza virus enabling virus to be prepared in the same way for use in both equine and hamster challenge studies. Results of an initial experiment mimicking the first equine challenge reported above, yielded similar results; namely the older the vaccine strain, the less effective the protection, assessed as infectious virus recovered from the lung (Table 20).

Subsequent studies will compare the protection afforded by American-like and European-like vaccines against American-like or European-like virus challenge. If the results obtained using the

**TABLE 20: Virus excretion from hamsters challenged with Sussex/89**

Group	Hamsters	
	% excreting virus	Mean log10 virus titre (±SE)
Unvaccinated control	100	4.4 ± 0.09
Miami/63	87	2.6 ± 0.05
Fontainebleau/79	53	1.4 ± 0.5
Kentucky/81	53	1.3 ± 0.44
Suffolk/89	13	0.1 ± 0.11
P-value*	0.001	0.0007

\*Significance of differences between vaccine groups (control group excluded) using ANOVA

hamster challenge model are in agreement with those obtained in horses, this model will be useful as an indicator of relative vaccine efficacy against emerging strains.

## DISCUSSION

### Question

What is the relevance of the studies comparing sera from different species for antigenic analysis to the situation in the field, ie in deciding when vaccine strains need to be updated?

**P. Yates:** Obtaining data on antigenic variation enables the identification of emerging strains which differ significantly from current vaccine strains and should therefore be subject to further testing, eg antigenic analyses using equine sera and experimental vaccination/challenge studies. We are seeking the most appropriate small animal sera which reflects the reactivity of equine sera and cross-protection in horses.

### Question

What are the levels of HI titres?

**P. Yates:** HI titres with guinea pig sera are generally low, whereas titres with both hamster and ferret titres are generally high.

### Question

Do sera from different animals yield similar HI titres?

**P. Yates:** There is some variation between individual sera.

### Question

What is the relevance of small animal sera differentiation to the situation in horses?

**P. Yates:** As before, an indication of significant antigenic variation that should be tested further. Because equine sera are cross-reactive without necessarily being cross-protective and bearing in mind the relative ease of obtaining sera from small animals compared with horses, small animal sera are preferred for assessment of antigenic variation.

**Comment:** Generally, ferret sera raised against American-like strains cross-react with European strains more strongly than the reverse situation (sera against European strains cross-reacting with American strains). This may be relevant to cross-protection studies.

**A. Hay:** If differentiation is seen in the serosurvey, this will be a warning of major antigenic divergence from existing vaccine strains likely to have a major impact upon vaccine protective efficacy. Thus, analysing post vaccination equine sera should continue as a means of identifying emerging isolates that carry the highest risk of escaping current vaccine protection.

**H. Townsend:** SRH levels correlate well with protection and may be more relevant than HI titres.

**M. Barrandeguy:** I agree with the benefits of SRH testing from our experiences with vaccinated horses.

### Question

How do the results with post infection equine sera compare with post vaccination?

**P. Yates:** Currently the available panel of such sera has been raised against a limited number of isolates, but so far no significant difference has been noted compared with post vaccination sera.

**T. Chambers:** Dr Lunn's group have previously reported on antibody isotype variation comparing post vaccination with post infection equine sera, I would like to ask Paul if this approach could be used to investigate varying potential efficacy of post vaccinated sera against current strains?

**D. P. Lunn:** The previous results were from naïve horses vaccinated with a particularly ineffective vaccine. It is unlikely that there would be similar differences in isotypes for current vaccines. It appears that American-like virus strains are predominating within Europe. Have European strains been isolated from American outbreaks?

**T. Chambers:** The only reported European-like isolate from America was from Canada (Sakatoon/90).

**D. Powell:** The numbers of horse shipments from the USA have increased dramatically over recent years.

### Question

What is the likely significance of sublineages within the American-like viruses, as reported by Dr Lai?

**A. Hay:** The level of divergence amongst American sublineages is probably insufficient to affect vaccine efficacy, it is much less than between, for example, Newmarket/1/93 and Newmarket/2/93.

**D. P. Lunn:** Is it true to say that, as American-like strains are predominating, they are the most important for inclusion in updated vaccines?

**J. A. Mumford:** That is the general observation and it is important that recent American-like strains are included in European vaccine formulations.

**D. P. Lunn:** Is it known whether current American vaccine formulations can be overcome by European-like challenge?

**J. A. Mumford:** This has not been directly studied yet. It could be argued that the increasing prevalence of American isolates in Europe is an indication that European vaccines (which generally include an updated 'European-like' strain, but have not been updated to include an 'American-like' strain) are effective at controlling European-like viruses, but show reduced effectiveness against American-like viruses.

**J. A. Mumford:** What recommendations should be made concerning revision of the criteria for strain selection? For example, should the analysis of post vaccination field sera be continued in light of the high level of cross-reactivity of such sera against antigenically distinct strains? Despite this cross-reactivity, it appears that there is a reduction in protective efficacy for vaccines comprising old strains against challenge with newer strains.

**A. Hay:** The serosurvey is still useful. Although differences are not currently apparent, emerging strains with significant antigenic differences may be found to show reduced reactivity against post vaccination field sera. This would be a clear indication of strains to be considered for inclusion in updated vaccines.

### Question

Should an alternative testing procedure be used, such as a comparative serum neutralisation assay? Such a test is technically more demanding and time consuming, but with the relatively few number of new equine influenza virus isolates currently being identified each year, it may be a practical option.

**J. A. Mumford:** Are there any volunteers to undertake further cross-protection/challenge studies in horses? For example, looking at isolates that show the most significant antigenic differences from analysis of cross-reactivity of ferret sera?

**H. Townsend:** We may possibly be able to do so, depending upon the likely costs.

**D. G. Powell:** Perhaps Dr Mumford could provide an experimental framework/estimate of costs for further equine challenge studies?

**J. A. Mumford:** Are genetic differences predictive of antigenic differences?

**A. Hay:** Genetic changes are useful as a guide. If a change occurs in more than one antigenic site this is likely to result in antigenic change. Changes in all 4 sites are indicative of significant antigenic change. Genetic variation is also useful for

tracking virus spread, which may indicate inadequate horse movement control or inadequate vaccine protection within particular countries. However, direct measurements of antigenic change are most important when identifying emerging variants for potential inclusion in vaccines. For this purpose post infection ferret (and possibly hamster) sera are the best available tools. Major antigenic variants identified in this way can then be studied further in vaccination/challenge studies. When considering recommendations for strains to be introduced into/removed from current vaccines, it is important to be conservative and have good reasons for change, based upon antigenic variation, current/predicted prevalence of emerging strains and evidence of vaccine breakdown in the field. In view of the degree of international movement of horses, strains included in vaccines should be relevant both to the region of origin and the likely regions of travel/export.



# SESSION 4:

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## Vaccines

*Chairman: B. Hardy*

*Rapporteurs: P. Morley and J. A. Mumford*

## CURRENTLY AVAILABLE VACCINES

The session on vaccines opened with a review of current products from manufacturers, representing the major manufacturers in North America and Europe. Vaccines included inactivated whole virus in the presence of an adjuvant (alhydrogel, aluminium phosphate, carbomer, or oil) and subunits (ISCOMS or micelles). Vaccine viruses were grown in eggs for all but one vaccine, for which viruses were cultured on Madin-Darby canine kidney (MDCK) cells. The European manufacturers had generally adopted single radial diffusion for the standardisation of antigen content, whereas American producers generally continued to use chick cell agglutinating units.

In general, American vaccines contained recent American isolates (eg Kentucky/92 or Kentucky/93) and European products contained recent European viruses (eg Suffolk/89, Borlange/91 or Newmarket/2/93). At the time of this meeting, no licensed products were available containing both recent American-like and European-like isolates despite the recommendations following the 1995 consultative meeting of WHO and OIE experts. However, the majority of the manufacturers reported that they

were in the process of updating strains to include recent isolates from each lineage. Many vaccines still contained the H3N8 prototype virus Miami/63 and some vaccines were relying on other epidemiologically irrelevant viruses such as Lexington/63, Fontainebleau/79, Brentwood/79 or Kentucky/81. All the vaccines contained representatives of H7N7; most included the prototype H7N7 strain, Prague/56, although 2 companies had included Newmarket/77 as the most recent H7N7 isolate. Several companies produced a range of multivalent vaccines, in which influenza was combined with other antigens such as equine herpesvirus types 1 and 4, eastern, western and Venezuelan equine encephalitis virus and tetanus toxoid.

Vaccination schedules required a primary course of 2 doses, 3–6 weeks apart, followed by boosters at 6–12 month intervals. It was generally recognised that, in many cases, such schedules would not maintain protective levels of antibody and more frequent administration was advised in high-risk situations. Some primary courses were recommended in foals from 3 months of age, others from 5 months of age.

## VACCINE CHARACTERISTICS AND PERFORMANCE - COMPARATIVE SEROLOGICAL STUDIES

### *H. Townsend*

An independent serological study was conducted in 1998 to compare equine influenza vaccines licensed in North America. It was recognised that outbreaks were common in vaccinated horses but there was little published information on antibody responses to existing vaccines, although duration of immunity was generally thought to be short-lived. The study was a triple blind, randomised field trial with serological monitoring of serum antibody responses following an initial and a booster vaccination, the decline in serum antibody concentrations following the last vaccination, and rate of adverse responses to vaccination.

Four of the 5 vaccines examined were killed virus, adjuvanted vaccines, 2 of which held the majority of the equine influenza vaccine market in

North America; the fifth vaccine was a subunit vaccine. Horses were randomised into vaccine groups, including a control group, and vaccinated at Weeks 0 and 4. Horses in the control group were given a saline placebo. Sera were collected at the beginning of Weeks 0, 4, 8 and 16. Antibody concentrations were measured by SRH and reported relative to a standard serum with a relative antibody concentration (RAC) of >44% defined as consistent with clinical protection.

No adverse responses occurred to any of the vaccines during the study, and the unvaccinated sentinels confirmed that no infection occurred during the trial. Although a proportion of animals began the study with high levels of antibody (RAC>44%), and were therefore unlikely to respond to vaccination, some important differences

in the responses of the groups of horses to the vaccines were observed. The response 4 weeks after the second vaccination to the 2 market-leader killed virus vaccines was modest. Only 50% of those horses receiving these 2 vaccines achieved protective concentrations of serum antibody. Furthermore, the response to the remaining 2 killed virus vaccines was poor and not significantly different from the saline placebo. The response of horses given the subunit vaccine was significantly higher than that of horses in the other vaccine groups and was equivalent to post infection levels, with 90% of the animals achieving protective levels. There was a precipitous decline in serum antibody concentration in all groups over the 12-week period following the second vaccination, and less than 30% of animals in any vaccine group had protective concentrations of antibody 12 weeks after the second vaccination. Antibody responses of horses within and between the vaccine groups were highly variable.

In conclusion, protective concentrations of serum antibody were short-lived, the killed virus vaccines were not effective in stimulating protective levels of antibody, and there was a clear indication of marked variation among vaccines and among individual horses. Although the study provided valuable independent data from a randomised and blinded trial, it was recognised that there were relatively few horses per group. It was recommended that more independent and comparative studies of vaccine efficacy are needed; further experimental challenge studies should be undertaken, and data arising from such studies should be made readily available to veterinary practitioners and horse owners.

### **A. Cullinane**

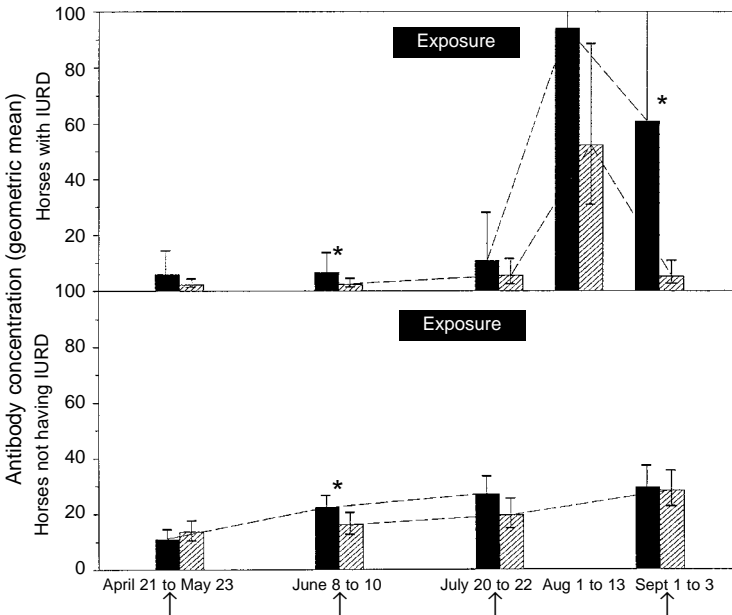
Data from 2 studies carried out at the Irish Equine Centre were presented. The first was a comparative serological study between 3 vaccines performed a few years earlier, since when some of the manufacturers have updated their virus strains, but the adjuvants have remained essentially the same. Prevac and Resequin (Hoechst) contained aluminium hydroxide as adjuvant and the virus strains Prague/56 (H7N7), and the H3N8 strains Miami/63 and Fontainebleau/79. Resequin also contained equine herpesviruses 1 and 4 strains and reovirus 1 and 3 strains. The third vaccine was Equip (Schering-Plough), which is an ISCOM vaccine and, at the time, contained Newmarket/77

(H7N7) and Brentwood/79 (H3N8). Groups of 7 unvaccinated Thoroughbred yearlings received one of the 3 vaccines in accordance with the Turf Club regulations (2 primary doses 6 weeks apart followed by a booster 6 months later). The horses were sampled each day they were vaccinated, 4 weeks and 4 months after their second vaccination, and 2 months and 4 months after their third vaccination. Four months after the completion of the primary course of vaccination, Prevac produced significantly higher antibody titres than either the ISCOM product or the multivalent vaccine Resequin. As Prevac and Resequin contained the same concentration of influenza antigen and the same adjuvant, it was concluded that a monovalent vaccine is preferable to a multivalent vaccine.

The vaccines used in the second study each contained a more recent European-like isolate. Two groups of 32 horses on the same premises received 2 doses of either Carbomer-adjuvanted vaccine (containing Prague/56, Miami/63 and Suffolk/89) or ISCOM vaccine (Newmarket/77, Brentwood/79 and Borlange/91) 4 weeks apart and a booster 6 months later. The sera were assayed by SRH for antibodies to Prague/56 (H7N7), and the H3N8 viruses Brentwood/79, Kildare/89 (European-like) and Kildare/92 (American-like). Both groups of horses had protective SRH antibody levels ( $\geq 120$  mm<sup>2</sup>) at the end of the study, ie 6 weeks after the third vaccination. The carbomer-adjuvanted vaccine stimulated a greater SRH response after the first dose of vaccine and stimulated a greater SRH response to Prague/56 and Kildare/89 throughout the trial. Unfortunately, due to dispersal, it was not possible to follow these horses for subsequent sampling.

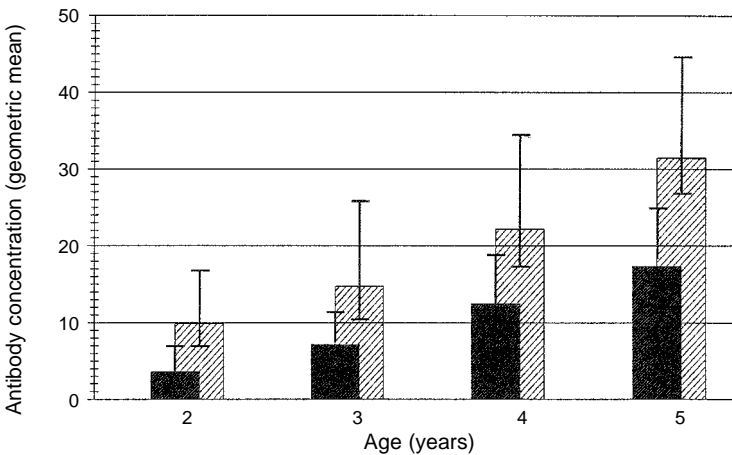
### **J. Traub-Dargatz**

A field study of serological responses to vaccination was carried out on 6 premises in Colorado between June 1997 and October 1998 and included 118 horses ranging from one to 26 years of age (mean age 11 years old). Horses on one of the premises had no previous history of vaccination, on 4 premises horses were vaccinated against influenza twice yearly, and on one 4 times yearly. The vaccine used was Rhino-Flu, SmithKline (Pfizer; this vaccine has subsequently been withdrawn from the market). Serology was determined pre-vaccination and 14 days post second vaccination against Prague/56 (H7N7), and the H3N8 antigens Miami/63 and Kentucky/81



Key: Geometric mean SRH values to Kentucky/81 (H3N8) among vaccinated horses (solid bars) and placebo-treated horses (hatched bars). Upper graph represents data from horses that developed IURD during an epizootic, lower graph represents data from horses not having IURD. Rectangles (exposure) identify the period of natural exposure. Arrows identify periods when horses received vaccine or placebo. Error bars represent 95% confidence interval, dashed lines identify significant ( $P < 0.05$ ) changes in antibody concentration between 2 sampling dates, asterisks indicate significant ( $P < 0.05$ ) differences between group means.

Fig 10: Mean SRH antibody concentrations before and after exposure to a natural outbreak of equine influenza.



Key: Geometric mean SRH values to Kentucky/81 (H3N8) among vaccinated horses ( $n=105$ ) of various ages before vaccination (solid bars) and 6 weeks after receiving a single dose of vaccine (hatched bars). Solid bars represent pre-vaccination antibody concentrations, hatched bars represent post vaccination antibody concentrations. Error bars represent bounds of 95% confidence intervals.

Fig 11: Mean SRH antibody pre- and post vaccination.

using the HI test. In response to the H7N7 vaccine antigen, 18% of horses with pre-vaccination titres  $< 1/16$  seroconverted but only 3% with titres  $> 1/16$  seroconverted. However, in response to the H3N8 vaccine antigen, only 2.5% of horses with pre-vaccination titres  $< 1/16$  seroconverted and none with titres  $> 1/16$  seroconverted.

**P. Morley**

The efficacy of a commercial vaccine was assessed in 462 Thoroughbreds stabled at a Canadian racetrack in a double blind, randomised, controlled field trial. Vaccine or saline placebo was

administered 4 times in the population at 6-week intervals. The vaccine contained 3 strains of inactivated influenza virus and inactivated equine herpesvirus type 4. A natural influenza outbreak occurred when 69% of the horses had received 2 doses of vaccine (at a mean of 58 and 19 days previously), and 31% had received one dose (mean of 19 days previously).

Vaccination of horses prior to the influenza epizootic did not result in a significant decrease in risk of respiratory disease. Severity of clinical disease was not different between affected vaccinated and control horses, but median duration

of clinical disease was 3 days shorter in vaccinated horses. No difference in mean antibody concentrations at the beginning of the study was detected between vaccinates and controls (Fig 10).

Increases in antibody concentrations after initial vaccination were small but statistically significant. However, serum antibody concentrations to H3N8 influenza viruses were lower prior to initial vaccination in horses that showed clinical signs during the epizootic ( $P=0.001$ ), and did not increase in these horses in response to vaccination. On average, affected

horses were younger (mean age  $2.6 \pm 0.1$  years) than horses that did not develop influenza ( $4.5 \pm 0.1$  years,  $P=0.001$ ). Upon arrival at the racetrack, older horses had higher antibody concentrations than young horses and developed better antibody responses to a single vaccination (Fig 11).

It was concluded that vaccination was of questionable benefit and it was recommended that further objective field evaluations of commercially available vaccines are needed to document their efficacy accurately.

## EFFECT OF MATERNAL ANTIBODIES ON THE RESPONSE TO VACCINATION

### *D. Wilson*

The widely recommended age for the onset of primary vaccination in foals is still 3–4 months. However, several studies have now documented lack of response to influenza vaccination in foals from vaccinated mares if they are vaccinated before 6 months of age. It is also suggested that early vaccination may render foals tolerant to later vaccination, resulting in poor response. In 1995/1996, 31 foals from mares boosted during the last 4 weeks of gestation against influenza, tetanus, equine herpesviruses 1 and 4, eastern equine encephalitis and western equine encephalitis were monitored. Group 1 (16 foals) received 3 vaccinations from 3 months of age (12, 16 and 26 weeks). Group 2 (15 foals) received 3 vaccinations from 6 months of age (26, 30 and 40 weeks). In Group 1 no foals seroconverted to 2 doses and only one seroconverted after 3 doses (ie at 6 months of age). In Group 2, only 2 foals had seroconverted after 2 doses (7 months of age) but a total of 7 had seroconverted by 9 months of age. Overall, many foals without maternal antibodies detectable by HI failed to respond to vaccination. It was concluded that maternal antibodies inhibit serological responses in foals (at least up to 6 months of age) to inactivated antigens in conventional intramuscular vaccines. Some individual foals are good responders but others respond less well. In some cases, although no response was detectable in the HI test, antibody was detectable by ELISA. In light of these results, the poor responses found in some field trials could be associated with early

vaccination causing an inhibited response to vaccination later in life. This suggests that foal vaccination programmes should be revised.

### *A. Cullinane*

HI assays were used to investigate the extent of interference of maternal antibodies with the humoral response to vaccination against equine influenza. Twenty-six foals born to mares that had been vaccinated 4 to 6 weeks prior to parturition were first vaccinated at 3 months of age and monitored serologically over a 12-month period. Only 2 of the 26 had detectable antibodies against H7N7 and H3N8 influenza viruses when tested 6 weeks after the second booster. They were re-vaccinated 4 times during the following 10 months but only the last 2 inoculations elicited a significant response. Foals born to the same mares in the following year were monitored to determine the rate of decline of maternal HI antibodies. At 3 months of age many still had significant titres but these had virtually disappeared at 6 months. Two vaccinations after the maternal antibodies had waned resulted in a detectable antibody response in only 4 of 26 foals. However, 22 foals responded to the third vaccination given 6 months later, and the mean HI titre against H3N8 viruses was higher than that of the foals born the previous year after 6 vaccinations. These findings suggest that foals born to vaccinated mares should not be vaccinated until the maternal antibodies have declined. Furthermore, it appears that vaccination in the face of maternal antibodies interferes with the development of active immunity

and specific immunological memory and may also inhibit the response to later vaccination.

**R. Holland**

Our studies have indicated that first vaccination in foals as late as 7 months of age failed to induce

seroconversion. We therefore advise that vaccination should begin at 9 months of age or later and that a course of 3 doses of vaccine should be given. The HI test is not sensitive enough for accurate detection of maternally derived antibody.

**DURATION OF IMMUNITY**

**A. Cullinane**

Results from earlier studies suggested that vaccination in accordance with the Turf Club rules leaves horses with low antibody levels for several months between the second and third vaccination. To examine whether an additional booster during that 6 month period would be beneficial, 24 Thoroughbred yearlings were divided into 3 groups of 8. Group A received an extra booster 3 months after the second vaccination, Group B received an extra booster 4 months after the second vaccination, and Group C were vaccinated in accordance with the Turf Club regulations with a 6 month interval between the second and third vaccinations. Six months after their primary vaccination, Groups A and B had higher antibody titres than Group C. These differences were even more marked 5 weeks after the third vaccination, with Group B having significantly higher titres than either of the other 2 groups. In summary, these findings suggest that horses such as Thoroughbred yearlings being prepared for sale would benefit from an additional booster between the primary course and the dose that is required by the Turf Club approximately 6 months later.

The duration of antibody response was studied in a group of Thoroughbreds that had

been vaccinated in accordance with the Turf Club rules ie 2 primary doses approximately 4 weeks apart and a booster 6 months later. The animals were vaccinated with an ISCOM-based vaccine, reputed to provide 15 months' protection after the third vaccination. Five months after the third vaccination the horses had negligible antibody titres against the Prague/56, Miami/63 and Newmarket/79 antigens. They were vaccinated at this time, and seroconverted within 4 weeks, but their antibody titres had declined significantly in 5 months and were extremely low 7 months after the fourth vaccination.

**N. Klein**

A study of duration of immunity was performed in influenza-naïve ponies vaccinated with an inactivated whole virus vaccine (Prevacun). The vaccine contained 2,000 HAU (50 µg HA) Prague/56(H7N7), and 2,000 HAU (20 µg HA) Miami/63 (H3N8) and Suffolk/89 (H3N8) adjuvanted with 9.0 mg aluminium hydroxide. Six ponies (Group A) received Prevacun S, and 6 ponies (Group B) received Prevacun ST, which contains tetanus toxoid (150 iu) in addition to influenza viruses. Vaccinations were given at around Weeks 0, 4 and 37. Following primary vaccination, SRH

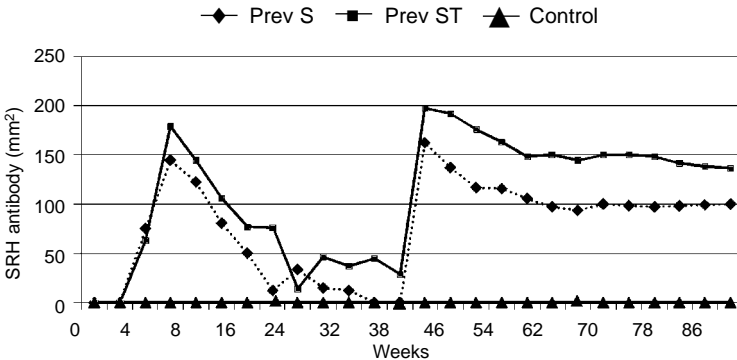


Fig 12: Mean SRH antibody (mm²) to Suffolk/89.

antibody levels to all 3 vaccine antigens declined such that at the time of the third vaccination the SRH antibody levels to H3N8 antigen were <50 mm<sup>2</sup> and the antibody levels to H7N7 antigen were <75 mm<sup>2</sup> (Fig 12). After the third vaccination, SRH (and HI) antibody levels were maintained at higher levels for a year after vaccination. Vaccinated ponies and 6 unvaccinated control animals were challenged one year after the third vaccination. Following challenge, coughing and mucopurulent discharge were not seen in vaccinates. The duration of pyrexia was significantly reduced in both vaccine groups and the peak titres and duration of virus excretion were significantly lower in both vaccine groups, compared with the controls. Updated Prevacun vaccines (Prevacun NN and NNT) contain the H3N8 strains Newmarket/1/93 and Newmarket/2/93.

**J. A. Mumford**

The duration of immunity provided by Duvaxyn IE Plus (Solvay Duphar BV) was assessed in a challenge study. Five ponies, which had received

3 doses of Duvaxyn IE Plus with the third dose one year before, and 5 unvaccinated controls were challenged with Sussex/89 (H3N8) virus. The ponies were monitored for clinical signs (Fig 13) and nasal swabs were taken daily for 10 days following challenge for virus isolation (Fig 14). At the time of challenge (one year after the third vaccination), all 5 vaccinated ponies had detectable SRH antibody to each of the 3 antigens contained in the vaccine, with the exception of one animal in which no antibody was detected against Miami/63. This pony also had the lowest levels of antibody to both Prague/56 and Suffolk/89. All but one of the 5 vaccinates seroconverted to the H3N8 viruses (Table 21). All 5 unvaccinated controls shed virus for at least 4 days after the challenge. The mean duration of shedding was 4.6 days. In contrast, virus was recovered from only 2 out of 5 vaccinates. One vaccinate shed virus for one day only. The other, which had the lowest level of pre-challenge antibody of the group, shed virus for 4 days, further supporting the relationship between antibody and protection.

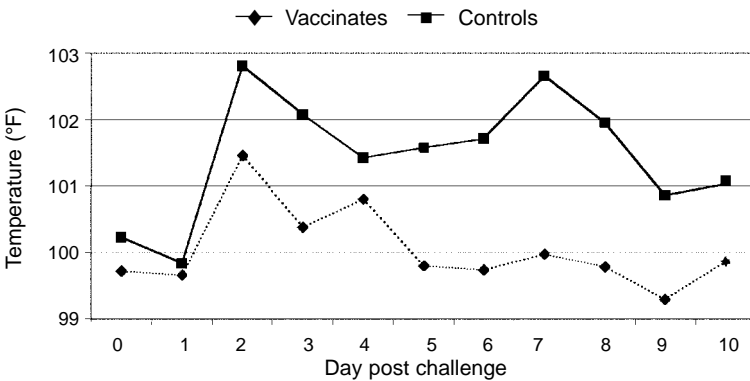


Fig 13: Mean temperatures of ponies challenged with Sussex/89 one year after the third dose of vaccine (Duvaxyn IE Plus).

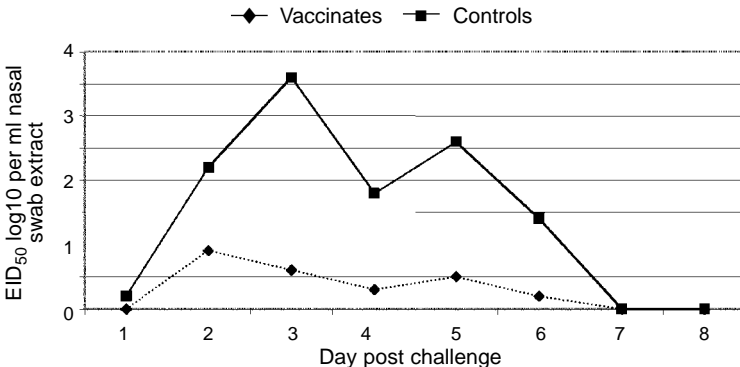


Fig 14: Isolation of equine influenza virus from nasopharyngeal swabs following challenge with Sussex/89 one year after the third dose of vaccine (Duvaxyn IE Plus).

**TABLE 21: Summary of responses to challenge with Sussex/89 one year after the third dose of vaccine (Duvaxyn IE Plus)**

Group	Mean pre-challenge SRH to Suffolk/89 (mm <sup>2</sup> )	No. ponies (duration in days)			Seroconversion
		Cough	Temperature	Virus isolation	
Controls	0	5/5 (4.2)	5/5 (4)	5/5 (4.6)	100%
Vaccinates	86.74	0/5 (0)	2/5 (1.5)	2/5 (2.5)	80%

## RESPONSES TO VACCINATION IN YOUNG THOROUGHBREDS

J. R. Newton

A field survey was conducted among 222 Thoroughbred yearlings that had recently entered 7 training yards in Newmarket, England, after the autumn sales in 1998. The aims of this study were to monitor field serological responses in young Thoroughbreds given a recently updated commercial vaccine, and to identify factors in the previous vaccination history that influence serum

SRH antibody levels in horses entering training. The vaccine used was Prevac ProTM (Hoechst Roussel Vet), which was then an inactivated virus vaccine adjuvanted with aluminium hydroxide, and containing Prague/56 (H7N7) and the H3N8 strains Miami/63 and Newmarket/2/93. Two doses (2 ml) of vaccine were administered by intramuscular injection 4 weeks apart.

**TABLE 22: Mean SRH antibody values at different levels of explanatory factors examined**

Factor	Level/category	Standardised SRH (mm <sup>2</sup> )		
		Pre-V1	at V2	Post V2
Overall	Mean	64.1	156.0	159.4
	Median	57.8	155.5	157.9
Sex	Female	65.8	156.7	162.3
	Male	63.4	155.4	156.2
<b>t-test P-value</b>		<b>0.76</b>	<b>0.81</b>	<b>0.22</b>
Previous vaccinations	0	41.0	142.7	161.1
	1-2	43.2	166.9	171.3
	3-5	82.3	157.7	156.3
<b>ANOVA P-value</b>		<b>0.0001</b>	<b>0.0068</b>	<b>0.12</b>
Previous carbomer vaccinations	0	46.7	151.4	160.0
	1-2	74.8	163.6	161.7
	3-4	80.4	156.5	159.0
<b>ANOVA P-value</b>		<b>0.0002</b>	<b>0.18</b>	<b>0.93</b>
Previous vaccine types given	Mixed types	97.4	163.7	155.7
	All carbomer	70.2	158.1	161.5
	All other type	55.3	161.5	156.6
	No previous	41.0	142.7	160.9
<b>ANOVA P-value</b>		<b>0.0001</b>	<b>0.035</b>	<b>0.83</b>
Time from last vaccination	6 months	89.4	160.3	159.7
	> 6 months	47.5	158.2	159.4
<b>t-test P value</b>		<b>0.0001</b>	<b>0.71</b>	<b>0.95</b>
Age at first vaccination	6 months	47.2	155.7	150.1
	> 6 months	68.0	155.5	162.8
<b>t-test P-value</b>		<b>0.028</b>	<b>0.98</b>	<b>0.037</b>

**TABLE 23: Multiple linear regression modelling**

Factors in the model	Parameter estimate	P-value
Intercept	45.4	
Time since last vaccination	-26.6	0.0051
No of previous vaccines	21.8	0.0026
Previous vaccine type	-13.4	0.0445
Age at first vaccination	20.8	0.0499

Serum antibody levels were determined by SRH against the Newmarket/2/93 strain. Test results were standardised to reduce between-test variation. A number of independent variables was considered including sex, number of previous vaccinations, previous vaccine types, number of carbomer-adjuvanted doses, time since last vaccination and age at first vaccination. Significance testing of the null hypothesis that there was no difference between the mean SRH levels at the different levels/categories of individual factors was by univariate analysis. Student's *t*-test was used for factors with 2 levels or categories and one-way analysis of variance (ANOVA) for factors with more than 2 levels or categories.

Multiple linear regression analyses were used to develop predictive models of the relationship between SRH antibody levels and likely

explanatory factors based on the results of univariate analyses. Stepwise model building identified factors that were considered to have significant predictive power for SRH antibody levels in yearlings when they entered training.

There was wide variation in SRH antibody values in yearlings at the time of entry into training yards in the autumn, and mean SRH antibody values were considered to be non-protective (Table 22). There was a high response to the last vaccination but little additional response to a second dose. The number of previous vaccines administered, previous vaccine types administered, time since last vaccination, and age at first vaccination were all significantly predictive in determining levels of SRH antibody in yearlings at the time they entered training.

In conclusion, factors in the previous vaccination history are important in determining the levels of protective SRH antibody at the time that young horses enter training and, consequently, are likely to affect the risk of introducing influenza infection. Administration of influenza vaccination in yearlings soon after entering training produces protective levels of antibody and overrides the effects of these earlier factors. Vaccinating yearlings and their contacts several weeks before moving them to the autumn sales and training yards could protect them against infection.

## PROTECTION AGAINST EXPERIMENTAL INFECTION - CORRELATES OF IMMUNITY

J. A. Mumford

In initial experimental challenge studies, protection against influenza infection was correlated with levels of SRH antibody (Fig 15). However, protective levels (against seroconversion) varied with challenge techniques and infectious viral doses (Table 24). The European Pharmacopoeia and the OIE manual now accept the nebulised aerosol challenge method for demonstrating efficacy of European influenza vaccines.

### REFERENCES

Mumford, J.A., Jessett, D., Dunleavy, U., Wood, J., Hannant, D., Sundquist, B. and Cook, R.F. (1994) Antigenicity and immunogenicity of experimental equine influenza ISCOM vaccines. *Vaccine* **12**, 857-864.

**TABLE 24: Protective levels of antibody measured by SRH in different experimental challenge experiments**

Challenge technique	Infectious viral dose (EID <sub>50</sub> /ml)	Protective SRH threshold (mm <sup>2</sup> )
Contact exposure	24 h contact	70
Intranasal instillation	5 ml x 10 <sup>8</sup>	65
	5 ml x 10 <sup>8</sup>	74
	5 ml x 10 <sup>7.3</sup>	84
Nebulised aerosol	20 ml x 10 <sup>6.0</sup>	120
	20 ml x 10 <sup>6.6</sup>	128
	20 ml x 10 <sup>7.6</sup>	154

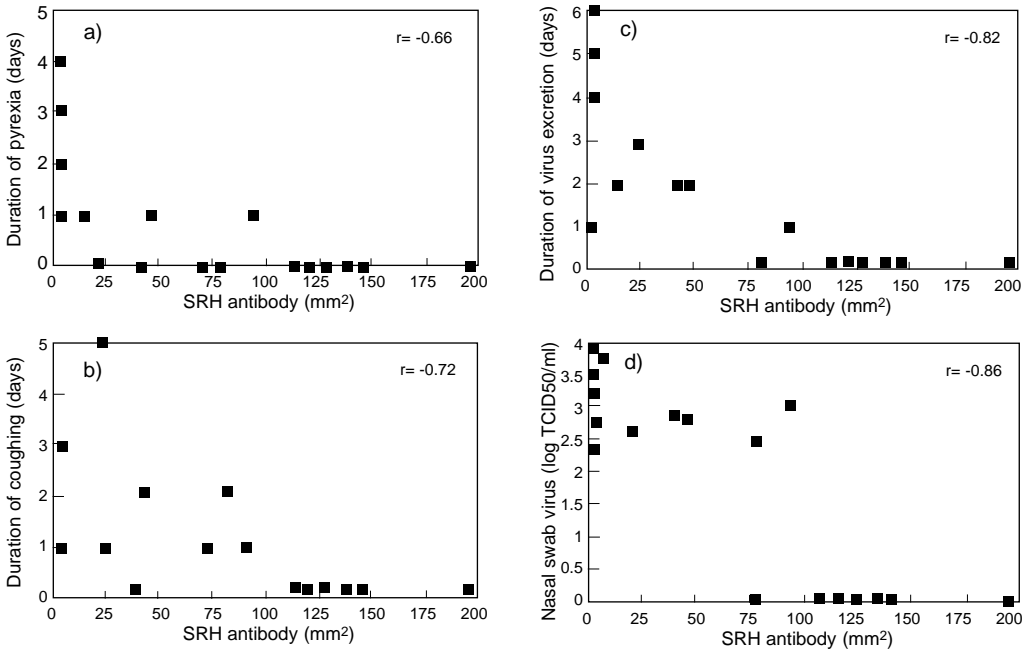


Fig 15: Correlation between clinical signs after challenge infection and the pre-challenge level of SRH antibody; a) duration of pyrexia, b) duration of coughing, c) duration of virus excretion, d) amount of virus excretion.

## PROTECTION AGAINST FIELD INFECTION

### United Kingdom (J. R. Newton)

In 1995, blood samples were taken from 25 Thoroughbred racehorses in a yard in Newmarket 10 days before influenza was diagnosed. Convalescent blood samples were taken 3 weeks later. Nasopharyngeal swabs were taken from 11 horses at the time of influenza diagnosis. Blood samples were tested for SRH antibody to Suffolk/89, and influenza diagnosed on the basis of either a positive swab nucleoprotein ELISA or seroconversion between paired blood samples indicated by an increase in SRH antibody of  $\geq 25$  mm<sup>2</sup> (Table 25). An H3N8 subtype virus was isolated and characterised as European-like.

During April 1998, acute blood samples were taken from 52 Thoroughbred racehorses in a Newmarket racing yard, and convalescent blood samples were taken 2 weeks later. Nasopharyngeal swabs were also taken from 21 horses at the time of influenza diagnosis. Blood samples were tested for SRH antibody levels to Newmarket/2/93 (H3N8). The basis of influenza diagnosis was the same as in

1995 (Table 26). An H3N8 subtype virus was isolated (Moulton/98) and characterised as American-like.

Mean SRH levels were the same in both outbreaks (172 mm<sup>2</sup>) among horses that did not subsequently seroconvert (Table 27). However, in 1995, no horses seroconverted if they had pre-exposure SRH antibody levels of  $\geq 150$  mm<sup>2</sup> whereas, in 1998, seroconversion was detected in horses with higher than 150 mm<sup>2</sup> SRH concentrations. Further, there was evidence of infection among a large proportion of horses in 1998 which, based on extrapolation from the 1995 outbreak data, should have been protected.

In a typical European-like strain outbreak in 1995 there was a clear threshold of protection from serum SRH antibody, which correlated closely with the protective levels determined in experimental challenges of ponies vaccinated with strains homologous to the challenge virus. In an American-like strain outbreak in 1998 there was no clear threshold of protection produced by

**TABLE 25: 1995 Newmarket field outbreak results**

ID	SRH (mm <sup>2</sup> )	Serology	NP ELISA
1	0	+	
2	51	+	
3	65	+	
4	76	+	+
5	85	+	+
6	86	+	
7	103	+	
8	108	+	-
9	108	+	+
10	120	+	+
11	120	+	+
12	122	+	
13	126	-	

ID	SRH (mm <sup>2</sup> )	Serology	NP ELISA
14	130	+	-
15	132	+	+
16	140	+	
17	140	-	
18	149	+	-
19	165	-	-
20	174	-	
21	182	-	-
22	182	-	
23	184	-	
24	196	-	
25	199	-	

**TABLE 26: 1998 Newmarket field outbreak results**

ID	SRH (mm <sup>2</sup> )	Serology	NP ELISA
1	34	+	+
2	71	+	
3	78	+	+
4	85	+	
5	86	-	
6	86	+	+
7	86	+	-
8	88	+	
9	93	+	
10	99	+	
11	100	+	-
12	108	+	+
13	110	+	-
14	112	+	-
15	118	+	
16	120	+	
17	122	+	+
18	124	+	+
19	124	+	+
20	128	+	
21	130	+	
22	132	-	
23	134	-	
24	134	+	
25	140	+	
26	145	+	

ID	SRH (mm <sup>2</sup> )	Serology	NP ELISA
27	147	+	+
28	147	+	
29	147	+	
30	153	+	+
31	153	+	-
32	156	+	+
33	156	-	
34	160	-	
35	160	+	
36	160	-	-
37	163	-	
38	170	+	
39	174	-	
40	179	+	
41	179	+	
42	184	+	-
43	184	-	-
44	189	-	
45	194	+	
46	194	-	-
47	196	-	-
48	201	-	
49	201	-	
50	212	-	
51	217	+	+
52	217	-	

vaccination, which is consistent with observations from the 1989 UK outbreak. It was concluded that vaccines containing only a European-like virus did not provide adequate protection against challenge with an American-like virus, suggesting the need to include an American-like strain in UK-licensed vaccines.

**Sweden (B. Klingeborn)**

Vaccination has not been compulsory in Sweden since 1993. During an outbreak of influenza in

Sweden in 1998/1999, the incidence of disease was  $\geq 20\%$  and, in 11% of stables, severe clinical signs were detected, compared with 4.5% in the 1992/1993 outbreak. An American lineage virus was isolated and the Swedish vaccines only contain a European variant. It was noted that the more severely affected racetracks generally had older horses. The current vaccination policy is 2 primary vaccinations and thereafter 6 monthly until 4 years of age, then annually. Since this outbreak, policy has shifted from isolation of

**TABLE 27: Summary of field outbreak investigations in 1995 and 1998**

Parameter	Seroconversion*	1995	1998
H3N8 vaccine strain		Suffolk/89 (European-like)	Suffolk/89 (European-like)
Last vaccination		6 months	5 months
Vaccination history		Mixed types	All carbomer
Outbreak strain isolated		Newmarket/95 European-like	Moulton/98 American-like
Mean pre-infection SRH	-	172.0 mm <sup>2</sup>	172.4 mm <sup>2</sup>
	+	99.7 mm <sup>2</sup>	128.4 mm <sup>2</sup>
t-test P-value (H0:difference=0)		<0.0001	0.0003
Median pre-infection SRH	-	182 mm <sup>2</sup>	179 mm <sup>2</sup>
	+	108 mm <sup>2</sup>	126 mm <sup>2</sup>
SRH inter-quartile range	-	165–184 mm <sup>2</sup>	158–198.5 mm <sup>2</sup>
	+	80.5–126 mm <sup>2</sup>	99.5–153 mm <sup>2</sup>
SRH complete range	-	126–199 mm <sup>2</sup>	86–217 mm <sup>2</sup>
	+	0–149	34–217

\*SRH values analysed separately for horses that seroconverted (+) and those that did not seroconvert (-)

whole racetracks to the isolation of infected stables only; the stable is released from isolation 5 days after the last case has recovered. Although 5 days is short, this policy does seem to be effective.

### **Hong Kong (D. Powell)**

An outbreak of equine influenza in Hong Kong in 1992 closed racing for 7 days. The situation in Hong Kong is unique in that 1,000 horses are stabled on 3 levels over 40 acres. The annual booster (Duvaxyn IE containing Prague/56, Miami/6, and Kentucky/81) is given in June during

the off-racing season. In November 1992, the whole horse population was exposed to influenza because of the importation of horses from UK and Eire. HI responses were analysed using a homologous Hong Kong isolate and, because of the regular blood sampling and storage at the Hong Kong track, pre-exposure bloods were readily available. The results indicated that HI titres were generally lower for affected horses compared with those that did not show clinical signs. This suggests that if horses develop antibody they will be protected, but the need is for appropriate vaccines to achieve this.

## **NOVEL APPROACHES TO VACCINATION**

The session on vaccines concluded with 3 presentations on novel vaccination strategies, which aim to provide more efficacious vaccines.

### **DNA vaccines (D. P. Lunn)**

Immunity to influenza is influenced by systemic and mucosal immunity, the systemic response being representative of a TH1 response, whereas the mucosal immunity is more of a TH2. Natural infection results in mucosal IgA, and serum IgG<sub>A</sub> and IgG<sub>B</sub> responses. Vaccination with traditional vaccine (eg Fluvac) did not show increase in serum IgG<sub>A</sub> and IgG<sub>B</sub> but did show a large

increase in IgGT. DNA vaccination results in the *in vivo* synthesis of antigenic proteins in a manner very similar to that occurring in natural infection. Ponies were protected from challenge 50 days after DNA vaccination. A new vaccination that increases nasal IgA and serum IgG<sub>A</sub> and IgG<sub>B</sub> should be investigated. A plasmid expressing equine interleukin-6 (IL-6) was incorporated recently into the vaccine prototype because IL-6 is known to have a role in IgA stimulation. However, IL-6 only caused immunomodulation in the systemic compartment, ie had a positive effect on IgGT but no effect on IgA after challenge.

**Cold-adapted modified live intranasal vaccine (D. P. Lunn)**

Safety and efficacy studies were reported for a cold-adapted intranasal vaccine derived from Kentucky/91 virus (Heska). Challenge studies demonstrated that a single dose of vaccine induced significant protection against experimental challenge 3 months after vaccination in terms of suppression of clinical signs and reduction in duration of virus excretion.

In a safety study, influenza-naïve ponies were given a single dose of cold-adapted modified live intranasal vaccine. One group was simply vaccinated and the other group was vaccinated and then exercised on a treadmill for 5 consecutive days in order to induce immunosuppressive stress. Both groups and a group of unvaccinated control ponies were challenged 90 days after vaccination with a homologous virus. Severe clinical signs were produced in naïve control animals (Table 28), but no clinical signs were observed in either vaccine group. The vaccine provided solid clinical protection despite low increases in serum antibody titre.

**Recombinant vaccine (M. Aaby)**

A recombinant equine herpesvirus type 4 (EHV-4) and equine influenza virus vaccine has been under development in conjunction with Hoechst Roussel Vet. EHV-4 was selected as a backbone or vector vaccine because it has a large genome suitable for gene insertions, its host range is limited to equids and it is, itself, an important respiratory pathogen in horses. The EHV-4 vector had 3 gene deletions, and the HA and NA genes of equine influenza were

**TABLE 28: Clinical signs and viral shedding following challenge of ponies vaccinated with a live attenuated virus**

Group	No. ponies	
	Clinical signs	Viral shedding
Vaccinates	0	3
Vaccinates /exercised to induce immunosuppression	0	4
Controls	4	4

inserted at the deletion in glycoprotein E. Preliminary data suggested that an EHV-4 vector expressing either Prague/56, Miami/63 or Kentucky/81 genes can induce significant levels of antibody against equine influenza HA and reduce duration and severity of clinical disease. Immunogenicity and safety studies were conducted to investigate the possible excretion of vaccine virus from vaccinated animals, transmission of vaccine virus to non-vaccinated sentinel animals and persistence of latency of vaccine virus in vaccinated animals. A study of 19 horses vaccinated on Days 0 and 42 was terminated on Day 70. Nasal, urogenital and faecal swabs were taken and horses were subjected to post mortem examination. Various tissues, including trigeminal ganglia, were collected and PCR was used to look for wild-type virus and vaccine virus. No virus was isolated from swabs or cultivation of trigeminal ganglia and all horses were negative on PCR to vaccine virus. However, wild-type virus was detected on PCR.

**DISCUSSION**

**J. A. Mumford:** A comment on the study presented by Dr Townsend. The significance of differences between the American vaccines and one European vaccine is difficult to assess unless the relative efficacy of the European vaccine is established.

**K. Hennessy (Bayer) and J. Whalen (Fort Dodge):** More up-to-date vaccines were available (in USA) at the time of the study.

**J. A. Mumford:** Do vaccine manufacturers make changes to vaccines that are not reported to users?

Also, how confident are they about batch potency testing?

**N. Klein:** EU companies must comply with European Pharmacopoeia regulations, which requires antigen content of vaccines to be standardised by SRD or CCA so there is not much batch variability. For example, with the SRD test there is a requirement to show  $\geq 15 \mu\text{g}$ . Potency tests are required using guinea pig response to vaccination and perhaps we should use the response to dilution in guinea pigs.

**Comment**

In the USA currently there is no required HA content so different companies are allowed to use different influenza antigen levels. Production is not standardised but must be specified at the time of licensing.

The introduction of the SRD requirement has increased the HA content of vaccines but we must be careful when attempting to apply it to subunit or micelle vaccines.

*A. Cullinane:* Multivalent vaccines produce lower responses, which has also been seen by other workers including Dr Mumford.

**Comment**

Tetanus usually has an adjuvant effect.

*D. Wilson:* The trend in US vaccines is to reduce volume of dose and increase the number of antigens (to as many as 6 in a vaccine), this could lead to interference.

**Comment**

This is true, particularly for MLV.

*D. Wilson:* It may not be the number of antigens but the configuration of the antigen molecules.

*H. Townsend:* We ignore the epidemiology and risk period of disease when we use multivalent vaccines.

*D. Wilson:* I agree, horses are very often not at risk at the time that these multivalent vaccines are applied.

**Comment**

Adding an additional vaccine may not be needed if you reduce the time between second and third vaccinations. Are there any data to support this?

*J. A. Mumford:* There are supporting data.

*H. Townsend:* Influenza rarely occurs on studs, so why are we vaccinating foals that are not at risk?

*D. Wilson:* I agree that this is unnecessary.

*J. A. Mumford:* There is an advantage to starting vaccination in foals. By the time horses enter training as yearlings they will have received 3 doses of vaccine and subsequent responses should be more durable during a high risk period.

*P. Morley:* The absence of published information on efficacy is critical. We should require manufacturers to publish information in order that veterinarians can evaluate this instead of relying on marketing material. The assurance of several manufacturers that their products are satisfactory because they have passed licensing is not very reassuring in North America.

# SESSION 5:

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## Regulatory issues

*Chairman: A. Hay*

*Rapporteurs: B. Hardy and D. P. Lunn*

## OIE STANDARDS

J. A. Mumford

The OIE has played a major role in efforts to develop a system for updating vaccines in response to newly emerging strains of equine influenza virus and to provide standards for efficacious vaccines. One of the main objectives of the OIE is to provide guidelines and standards for health regulations applicable to international trade in animals. In pursuing this activity, the OIE produced the International Animal Health Code, the first edition of which was published in 1968. The Manual for Diagnostic Tests and Vaccines, which is compiled by the Standards Commission, is the companion volume to the Code and provides a wealth of internationally agreed essential scientific and technical information that complements the Code's trade provisions. The Manual, however, is not limited to those diseases for which the Code prescribes tests and biological products but covers all of the OIE Lists A and B diseases, and some additional diseases of importance. The equine influenza chapter of the manual was revised in 2000.

The OIE standard for equine influenza (a list B disease) relates to inactivated vaccines with or without adjuvant. Requirements are set out for relevant strains to be contained in vaccines and recommends against Miami/63. Single radial diffusion is recommended for quantitating antigenic content of vaccines, and single radial haemolysis to measure the antibody response to vaccination in horses is recommended with a peak value after 2 doses of 150 mm<sup>2</sup> regarded as indicative of a protective response.

The European Pharmacopoeia Monograph, also revised in January 1997, has similar recommendations to the OIE standard, but recommends only 85 mm<sup>2</sup> as a protective level of SRH antibody, which is certainly too low. Attempts to establish international harmonisation are ongoing.

### EUROPEAN PERSPECTIVE

#### *European Pharmacopoeia Monographs (J. A. Mumford on behalf of P. Castle)*

The European Pharmacopoeia Monograph equine influenza vaccine (inactivated) (1998:0249) is the

official standard in the 27 member countries (Austria, Belgium, Bosnia and Herzegovina, Croatia, Cyprus, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, the Netherlands, Norway, Portugal, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, 'the Former Yugoslav Republic of Macedonia', Turkey and the United Kingdom of Great Britain and Northern Ireland).

A revised version of the Monograph was published in 1997 and became official on 1/1/1998. The OIE meeting on equine influenza held in Newmarket (September 1995) came at an opportune time for work on revision of the Monograph. At the meeting, the need for regular review of the strains included in vaccines and for adaptation of regulations to facilitate introduction of newly recommended strains were recognised. These recommendations were taken into account before the revision was finalised, particularly as far as the introduction of new strains in an existing vaccine formulation is concerned.

The main objectives of the revision were to improve standardisation of vaccines and to provide a regulatory framework for the introduction of new strains. Compared with the previous Monograph, the major changes are:

- addition of a requirement for standardisation of the vaccine on the basis of haemagglutinin content, determined by a suitable immunochemical method such as single radial immunodiffusion;
- addition of details of the inactivation test on the harvest;
- addition of a reference to the OIE annual review of strains based on epidemiological evidence;
- addition of details of development safety testing;
- addition of detailed specifications for development immunogenicity testing; challenge testing horses is required for at least one of the vaccine strains where there is epidemiological evidence on correlation of antibody levels with protection for the other

strains in the vaccine, challenge tests in horses are not required; routine testing of batches is based on serology in guinea pigs; detailed methods for determination of antibodies by single radial haemolysis or HI are included;

- previously for batch safety testing, 2 doses were given simultaneously at separate sites followed by a single dose 14 days later; in the new Monograph, the double dose is given at a single site.

For the serological potency tests described in the new Monograph, a reference serum is required for each strain. Three reference sera corresponding to the most commonly used vaccine strains have been prepared by the European Department for the Quality of Medicines in collaboration with the Animal Health Trust and will soon be available\*. It was initially intended to assign for each serum a nominal titre and allowable tolerance for the SRH and HI tests. However, the collaborative trial to establish the sera has shown the clear superiority of the SRH tests over HI for titration of test sera and the initial plans may need to be changed to take account of these results. The Monograph may also need further revision.

Concurrently with the revision of the Monograph, for European Union member states, all of which are members of the European Pharmacopoeia, guidelines were drafted by the European Medicines Evaluation Agency (Committee for Veterinary Medicinal Preparations, CVMP) dealing with the licensing of equine influenza vaccines, particularly the introduction of new strains:

1. CVMP/116/96 Note for Guidance on Harmonisation of Requirements for Equine Influenza Vaccines: Specific requirements for substitutions of a strain (CVMP adopted July 1997).
2. CVMP/112/98 Note for Guidance on the Harmonisation of Requirements for Equine Influenza Vaccines: Specific requirements for substitution or addition of a strain or strains (CVMP approved November 1998).

These guidelines refer to the European Pharmacopoeia Monograph, they have similar objectives and the 3 documents are complementary. The full texts of the guidelines can be found on the agency's website (<http://www.eudra.org/>).

The European Monograph already represents a considerable degree of international harmonisation

for European countries, but it was clear from the meeting in Newmarket in 1995 that users of the vaccines would like to see wider harmonisation to give a guarantee of quality, safety and efficacy for vaccines in view of the extensive travelling undertaken by racehorses. At present there is no forum for such harmonisation. The Veterinary International Conference on Harmonisation might be an appropriate forum and may consider equine influenza vaccines for a future work programme.

***CVMP Fast Track Licensing System for harmonisation of requirements for equine influenza vaccines – specific requirements for substitution or addition of a strain or strains (P. P. Pastoret)***

The major aim of the CVMP Fast Track Licensing System guidelines is to facilitate updating of strains in accordance with annual publications by OIE of recommended strains. Two *in vitro* assays, SRD and SRH, for measuring antigen and antibody respectively allow this system to be put in place based on the published correlation between microgrammes of immunologically active HA in vaccines, antibody to HA stimulated by vaccines and protection afforded against infection. For licensing vaccines in Europe, the requirements of both European Union guidelines and European Pharmacopoeia Monographs must be fulfilled. Therefore attempts are being made to harmonise these. The European Agency for the Evaluation of Medicinal Products (EMA) in London is important for the evaluation of new biotechnology products. The CVMP, responsible for animal health, has 5 working parties on safety, pharmacovigilance, quality, efficacy and immunologicals.

The immunologicals working group of the CVMP/EMA were responsible for developing the guidelines to produce better vaccines suited to epidemiological situations, to speed up authorisation and to diminish the number of animals needed for development. The guidelines are intended for substitution of strains and addition of strains. The guidelines only refer to antigenic drift, not shifts. It is not expected that manufacturers will exclude H7N7 currently, or add 2 new strains for the same subtype. No decrease is anticipated in antigenic content, change to method of production or quantity of adjuvants. A 2-fold approach for testing new formulations is adopted. Data and documentation from the original dossier

\*Editor's note: these are now available

would be accepted when unchanged and, where necessary, the original dossier would be amended. Efficacy testing of vaccines with substituted strains relies on in-process monitoring of the HA content by SRD, followed by immunisation of guinea pigs and horses with final produce and measurement of antibody responses using SRH. Comparison of these responses with those achieved with the original vaccine demonstrated equivalent efficacy. This approach has obviated the need for challenge studies. To speed up the process, post authorisation commitments (ongoing trials and field monitoring of vaccines) could be used to verify lack of adverse reactions and immunogenicity of the vaccine.

## AMERICAN PERSPECTIVE

### *Current regulations and draft standard requirements for influenza vaccines* (R. Miller)

New standard requirements for influenza vaccines are in draft form after 5–6 years of preparation and may soon go into Federal regulation.

The new requirements require that the immunogenicity of vaccine, prepared in accordance with the Outline of Production, should be established, by challenge of horses, for at least one component virus strain of each represented virus subtype. Strains of the same subtype can be included without further challenge studies. Thus, another virus strain of the subtype may be included initially, added later, or substituted if immunogenicity of the vaccine with respect to that strain is established as prescribed or by demonstrating, in either horses or guinea pigs, that the elicited serum HI titre for the strain is equal to or greater than the serum HI titre elicited by the strain that was evaluated by challenge. The use of guinea pig HI titres would be acceptable only if a satisfactory dose response relationship is shown in that species. At least one strain of each subtype that demonstrates immunogenicity must be retained in the vaccine. If the original strain for which challenge data were generated is removed, challenge must be performed with the remaining or substituted strain.

The new regulations require that at least 15 horses of the minimum age recommended on the label be used as test animals. Each horse should have a negative serum HI titre ( $<1/10$ ) for each virus fraction (strain) to be tested prior to the onset

of the challenge study. Each of at least 10 horses will be administered a dose of vaccine by the recommended route. If recommended, a second dose will be administered by the same route after the shortest interval indicated on the label. At least 5 horses will be held as unvaccinated controls. All horses will be challenged not less than 60 days after administration of the last vaccine dose, with a currently relevant strain of equine influenza virus in a manner acceptable to The Animal and Plant Health Inspection Service (APHIS) and observed daily for clinical signs of disease for at least 7 days. Clinical parameters shall be evaluated subjectively and shall be scored in a manner acceptable to APHIS. Horses shall be bled immediately prior to challenge and serum samples obtained. If, for the fraction being evaluated, controls are not still seronegative at the time of challenge, the study is inconclusive. If, in a valid study, a clinically significant and statistically significant ( $P<0.05$ ) difference in characteristic clinical signs and temperature between vaccinates and controls is not demonstrated during the observation period, the proposed vaccine is unsatisfactory.

Following challenge studies in horses that establish minimum HI titres associated with protection, duration of immunity shall be established by demonstrating persistence of titre in a new set of horses. This study will be used to establish recommendations for re-vaccination with a given product.

The new regulations also require that the strains of virus used in the manufacture of equine influenza vaccines will be identified on the final container and carton labels.

### *Current testing and draft Supplemental Assay Method for equine influenza vaccines* (P. Foley)

The APHIS Veterinary Biologics programme is run by the Center for Veterinary Biologics (CVB). The CVB implements the provisions of the Virus-Serum-Toxin Act to ensure that the veterinary biologicals available for the diagnosis, prevention and treatment of animal diseases are pure, safe, potent and effective. It uses scientific information and regulatory processes to assure its customers that the veterinary biologicals products available for use in the USA are of high quality. It encourages open communication with stakeholders as new products and standards are developed. It hopes to provide leadership in the development and

**TABLE 29: Panel of virus strains for master seed virus testing**

H7N7	H3N8	
Prague/56	Miami/63	Kentucky/94
	Kentucky/81	Kentucky/97
	Alaska/91	Puerto Rico/97
	Jilin/89	Kentucky/98
	Newmarket/1/93	California/98
	Newmarket/2/93	New York/99

international harmonisation of standards for veterinary biological products.

Towards these goals, the CVB is formulating new standard requirements for immunogenicity and potency testing of inactivated equine influenza virus vaccines. Currently, potency testing of vaccine serials at the CVB laboratory is conducted primarily as outlined in the draft, ‘Supplemental Assay Method for Conducting the Hemagglutination Inhibition Assay for Equine Influenza Antibody’. For each vaccine under test, 10 guinea pigs are given half the dose given to horses on Day 0 and at 14–21 days. Serum is harvested 14–21 days after the second vaccination. HI tests are performed using untreated antigen with serum pre-treated with kaolin and chicken red blood cells. A test is valid if non-vaccinated controls remain seronegative and the working dilution of the test virus contains 4–8 HA units per 50 ml. The geometric mean titre (GMT) must not be less than the correlated GMT. If the GMT is less than the correlated GMT, a second test is run, and if the GMT of both tests is less than the correlated GMT, the vaccine is deemed unsatisfactory. Currently, 6 companies sell equine influenza vaccines in the USA; 4 use the HI potency test and the rest use plaque reduction virus neutralisation.

Serological identity testing to help determine antigenic relatedness of proposed master seed viruses and recent field isolates has recently been set up at the CVB laboratory. Antisera are being raised to a panel of viruses (Table 29) by intravenous injection of chickens. Serum is harvested 2 weeks later and a chequerboard HI performed. Additional strains will be added to the panel as needed, to promote the development of antigenically relevant equine influenza virus vaccines.

**Argentina (E. Nosetto)**

Argentina follows the recommendations of the OIE.

**Canada (H. Townsend)**

Generally, equine influenza vaccines registered for use in Canada have been previously registered for use in the United States. However, companies with vaccines registered in the USA must apply for Canadian registration if they want to market these vaccines in Canada. Generally the procedure involves inspection of documents submitted to the USDA by the Canadian authorities to ensure that they are satisfied with procedures undertaken to assure the safety and purity of the product. Rules similar to those used by the USDA would apply to a vaccine produced and marketed in Canada. Vaccines manufactured in countries other than the USA may be licensed based on document submission to the Canadian authorities but a risk assessment would be necessary because of concerns that such a product may contain organisms harmful to Canadian livestock.

**PROVISIONS OF STANDARDS**

**Standards for vaccine potency testing (U. Dunleavy)**

Equine influenza vaccine potency is measured both by *in vivo* and *in vitro* methods. The 2 *in vitro* methods widely used are the chick cell agglutination (CCA) test and the single radial diffusion (SRD) test. It has been shown that the assigned CCA values do not relate to the actual HA content of whole virus and split vaccines. As a result Europe and the USA have standardised human influenza vaccines by the SRD test since the early 1980s. The CCA is not suitable because it does not measure antigenic content but the antigen’s ability to agglutinate erythrocytes. The morphology of the virus present in the vaccine therefore effects the CCA titre. In addition the CCA test has poor reproducibility compared to the SRD test.

The SRD test was developed in 1975 and validated during the ‘swine flu’ vaccine trials of the same year. Reproducibility of the assay was confirmed in a collaborative study in 1981 and was shown to be within 10% between laboratories.

A typical SRD test consists of an agarose gel containing antiserum specific for influenza HA. Wells are cut into the gel and dilutions of the vaccine and reference antigen introduced. Precipitin rings are produced, the diameter of which correlates to the amount of antigen present. The HA content of the vaccine can then be

calculated by comparison with the reference preparation.

The SRD test was used to standardise equine influenza vaccines in the early 80s using Miami/63 and Prague/56 vaccines. The SRD test was validated clinically at the Animal Health Trust (AHT) using Miami/63 vaccine containing 1, 5, 15 and 50 µg HA in a 2 or 3 dose immunisation schedule. At least 2 doses of 50 µg HA were required to achieve 70% protection and 3 doses of 50 µg for >70% protection.

A collaborative study involving 15 laboratories was organised by AHT and NIBSC in order to check the reproducibility of the SRD test using Prague/56 vaccines. The %GCV was found to be in the order of 10% which is similar to that obtained with human influenza vaccines.

For the SRD test to work accurately, strain-specific reagents need to be readily available. NIBSC produces reagents for all the major human and equine (Table 30) influenza vaccine strains and has the facilities to produce reagents to any new vaccine strain, ensuring efficient, harmonised methodology of standardisation of equine vaccines worldwide.

**Standards for serological testing: European Pharmacopoeia collaborative study to assign values to reference antisera (P. Yates)**

In order to standardise serological assays performed in laboratories around the world, reference sera to equine influenza viruses have been produced and values assigned in a collaborative study. Ten laboratories participated in the collaborative study, 7 of which provided data. Each laboratory was supplied with freeze-dried preparations of the proposed reference sera (A, B and C), a freeze-dried negative serum (D) and 3 liquid test sera (E, F and G). Serum A was raised to the American-like H3N8 subtype virus Newmarket1/93, Serum B to the European-like H3N8 subtype virus Newmarket/2/93, and Serum

C to the H7N7 subtype virus Newmarket/77. Sera E, F and G were taken from horses that developed high antibody levels following experimental challenge with Newmarket/2/93 virus (E), medium antibody levels to Sussex/89 (F) and low antibody levels to Sussex/89 (G). Participants were required to carry out SRH and HI tests on all 7 sera in triplicate and on 5 separate occasions.

The variations in SRH antibody values are shown in Table 31. The range of replicate measurements differs greatly between laboratories, although the range with Sera A and B was below 10% for the more experienced laboratories (1, 2 and 5). Serum C was raised against a different strain to that used in the SRH assays (Prague/56), which may account for the increased variation with this serum. Variation between measurements is expected to be greater if they are carried out on different occasions. None of the laboratories were consistently able to perform assays within a range of  $\pm 10\%$ , although the most experienced laboratories (Labs 2 and 5) achieved a range of  $\pm 20\%$ .

There was large variation in the reproducibility of the assay between laboratories (up to 2-fold for Sera A and B and up to 4-fold for Serum C). The results of applying standard statistical methods to combine results from different laboratories depended on the method applied, due to the small number of laboratories involved and the large inter-laboratory variation. Therefore the approach adopted was to select a range of possible assigned values for each serum and to count number of measurements not deviating by more than  $\times$  per cent from this assigned value. This was carried out for  $\times = 10\%$ , 20%, 30%, 40% and 50%. From Table 32, it can be seen that an assigned value of 180 mm<sup>2</sup> for Serum A with a variation of  $\pm 20\%$  would yield valid results in most assays. Similarly, Serum B can be assigned a value of 155 mm<sup>2</sup>  $\pm 20\%$ , and Serum C a value of 125 mm<sup>2</sup>  $\pm 20\%$ .

**TABLE 30: Availability of equine influenza SRD reagents**

Virus strain	Antigen		Antiserum	
	Code number	Number of amps	Code number	Number of amps
A/eq/Prague/56 (H7N7)	85/553	850	84/673	700
A/eq/Miami/63 (H3N8)	87/510	1,200	84/677	1,100
A/eq/Kentucky/81 (H3N8)	85/520	950	85/516	1,200
A/eq/Newmarket1/93 (H3N8)	97/596	500	97/610	2,000
A/eq/Newmarket/2/93 (H3N8)	97/584	500	97/614	2,000

**TABLE 31: Variation in SRH results**

Lab	Variation (% ±)					
	Serum A		Serum B		Serum C	
	Within*	Between	Within	Between	Within	Between
1	6.3	8.8	7.9	10.0	16.3	24.0
2	8.1	8.4	3.7	16.0	10.2	10.2
3	16.3	17.1	16.5	23.6	11.4	13.1
4	34.7	42.6	23.3	39.8	27.3	63.9
5	4.7	9.7	4.9	14.8	9.3	10.7
6	12.2	29.0	8.0	28.6	ND	ND
7	25.2	25.2	33.0	35.0	20.1	72.2

\*variation within a test (triplicate) and between tests (5 occasions), ND = not done

**TABLE 32: Designation of SRH antibody value for reference Serum A using acceptance/rejection rates**

Lab.	No. of assays (out of 5) valid at each assigned value & allowed variation									
	170 mm <sup>2</sup>		175 mm <sup>2</sup>		180 mm <sup>2</sup>		185 mm <sup>2</sup>		190 mm <sup>2</sup>	
	10%	20%	10%	20%	10%	20%	10%	20%	10%	20%
1	0	2	0	3	2	5	2	5	3	5
2	0	5	2	5	4	5	5	5	5	5
3	1	5	1	5	3	5	5	5	5	5
4	3	3	1	3	0	3	0	3	0	3
5	5	5	5	5	5	5	4	5	4	5
6	0	4	1	4	1	4	1	3	1	1
7*	3	4	3	4	0	4	0	3	0	3
Total:	12	28	13	29	15	31	17	29	18	27

\* Laboratory 7 performed 4 assays, therefore total assays for all labs = 34

**TABLE 33: Variability of HI test**

Lab.	Most frequently measured titres						
	Serum A	Serum B	Serum C	Serum D	Serum E	Serum F	Serum G
1	128	128	64	<8	64	64	16<8
2	512	64	64	<8	512	32	64
3	256	512	256	8	512	256	128
4	160	40	80	<8	80	40	20
5	256	32	32	<8	64	32	8
6	32	32	nd	<8	64	16	4

nd=not done

**TABLE 34: Expressing SRH antibody levels of test sera (E, F, G) as relative antibody level of reference serum (B)**

Ratio	% variation					
	Week 1	Week 2	Week 3	Week 4	Week 5	Overall
E:B	6	4	6	7	3	5
F:B	8	1	2	5	4	6
G:B	7	6	4	3	1	7

Assigning an HI titre to the proposed reference sera proved more difficult because of the inherent variability of the assay. The observed ranges between triplicates varied by up to 8- and 16-fold. The most frequently occurring titres across weeks for each serum and laboratory are shown in Table 33. As can be seen, there is a large variation between laboratories. The geometric mean titres for each serum and the acceptable range were calculated as 128 (64–256) for Serum A and 64 (32–128) for Sera B and C. As the value and range assigned to the reference sera affects values achieved by commercial vaccines, and therefore the level of protection induced, the European Pharmacopoeia have proposed that the SRH test should gradually replace the HI test for testing vaccine efficacy.

This study confirmed that the SRH test is less variable than the HI test. The variation of experienced laboratories was shown to be around 20% but up to 72% in less experienced

laboratories. The SRH values that have been assigned to the proposed reference sera have been set with a range of  $\pm 20\%$ . However, it is felt that intra-laboratory variation could be reduced significantly with experience. It was suggested, therefore, that the variation is set at  $\pm 20\%$  for a trial phase and the results of assays from the users collated. The range of the sera could then be reduced to  $\pm 10\%$  if results permit.

An alternative method for assigning values to unknown serum is to express the antibody value as a proportion of the reference serum (Table 34). Such a calculation significantly improves the inter-laboratory variation (levels of variation for all laboratories less than 10%). Data from users will be evaluated to examine the validity of quoting serum antibody as relative antibody levels.

In conclusion, these reference sera are to be made available for standardisation of serological tests to support the Fast-Track Licensing System in Europe.

## DISCUSSION

**J. Minke:** The USDA draft document does not really move things towards international harmonisation (eg number of test animals required, challenge after 60 days as opposed to the 30 days required in European regulations).

**P. Morley:** If the HI test is so variable, can someone from the USDA explain why they have decided to use the HI in preference to the SRH test for measurement of anti-influenza antibodies?

**P. Foley:** The HI test was chosen because problems were encountered with the equipment used for measuring the SRH zones of lysis such that interpretation of results is rather subjective.

**Comment:** Scanning software could overcome this.

### Question

There is obviously a difficulty in measuring the antibody response to vaccines, so would the use of a virus neutralisation (VN) test be useful?

**A. Hay:** The VN test is useful for measuring specificity but not for quantitative analysis. There are also practical problems with using a VN test.

**P. Morley:** VN antibodies do correlate with SRH antibodies.

### Question

What is the timescale for the introduction of the new USDA regulations?

**R. Miller:** If the draft document were published in Jan 2000, there would be a 60-day comment period, then a 2–3 month revision period and then one year would be allowed for vaccine manufacturers to come into compliance.

**J. A. Mumford:** HI is so unreproducible that its use will not enable the USDA to achieve the standardisation they hope.

**P. Foley:** Other tests such as SRH could be acceptable provided companies validated them.

Various comments about lack of international harmonisation, but very slow progress in biologicals area.

**J. Minke:** A weakness of current killed vaccines is that the interval between 2nd and 3rd vaccine is too long. USDA propose challenge after 2 months, a shorter term might be sensible to test onset of immunity, and a later test for duration.

#### **Question**

What about regulations for new vaccination strategies such as DNA vaccination?

**P-P. Pastoret:** There are guidelines under development which will be based largely on the old

rules but will need changes relating to safety issues. The National Environmental Policy Act governs this and makes requirements of companies.

**J. A. Mumford:** Although this could be a contentious issue, we need standards for challenge studies, as there are many different techniques across different laboratories.

#### **Question**

How should the efficacy of the challenge infection be standardised?

**H. Townsend:** Blinded trials are essential.



# SESSION 6:

## International movement and disease control

*Chairman: D. Powell*

*Rapporteurs: J. R. Newton and T. King*

## OIE CODE AND STANDARD

J. A. Mumford

Safe international trade in animals requires a number of factors to be taken into account to ensure unimpeded trade, without incurring unacceptable risks to human or animal health. Because of the variations in animal health status in different countries, various options are offered in the OIE International Animal Health Code. Animal health status in the exporting country, the transit country(ies) and the importing country should be determined before selecting the requirements to be met for trade. To maximise harmonisation, veterinary administrations of member countries are encouraged to base their import requirements on the OIE standards, guidelines and recommendations set out in the International Animal Health Code.

Veterinary administration bodies, of 'equine influenza-free' importing countries require an

international veterinary certificate attesting that the animals are from an equine influenza free country or meet various conditions, eg that the animals were kept in isolation for 4 weeks prior to shipment, and showed no clinical sign of equine influenza in this period. No new animal should have been introduced to the facilities during this period and no animal in the isolation facilities should have shown clinical signs of equine influenza during the isolation period. The animals should have been vaccinated against both subtypes of equine influenza virus and should have received a boosted dose of vaccine not less than 2 or more than 8 weeks prior to shipment. The code for equine influenza is lacking in that no recommendations are provided for shipment of horses between countries which are not influenza free.

## REGIONAL REGULATIONS AND TRANSPORT ISSUES

### *Europe (J. R. Newton)*

The trade and importation of horses into the European Community (EC) is covered by various pieces of legislation known as the European Commission 'Directives' and 'Decisions'. There are no specific requirements regarding equine influenza in the trade of horses between EC members. This 'internal' movement of horses is reliant on veterinary inspections and health certification declaring freedom from signs of disease and no contact with other equidae suffering from infectious or contagious diseases in the previous 15 days. Non EC-member states or so-called third countries are grouped A to E according to their region in the world. Health certificates with appropriate tests and declarations are required according to the group from which the horses are being imported into the EC, particularly with respect to compulsory notifiable diseases (dourine, glanders, all types of equine encephalomyelitis including Venezuelan, equine infectious anaemia, rabies, anthrax, African horse sickness and vesicular stomatitis). There are, however, no specific requirements regarding

equine influenza except again for veterinary inspections and health certificates as per the movement of horses between member states of the EC. There are requirements that horses travel from third countries in pre-disinfected vehicles that do not allow droppings, litter or fodder to escape during transport.

### *USA (T. Cordes)*

No specific importation protocols exist with respect to prevention of equine influenza virus in the USA at the present time. Horses entering the USA are held at one of 3 points of entry and different quarantine protocols are employed according to the countries of origin in accordance with the notifiable disease status of these countries.

### *Canada (H. Townsend)*

Influenza in horses exported from Canada or imported into the country may be controlled, at least to some degree, through export and import standards. Horses leaving the country need a

current health certificate and a current negative Coggin's test, both signed by a federal veterinary inspector. Horses imported from the USA require a current health certificate and negative equine infectious anaemia test signed by the State veterinarian. Horses imported from any other country require a health certificate signed by the veterinary inspection service from the country of origin and must be seronegative to a variety of diseases including equine infectious anaemia, dourine, glanders, piroplasmosis and African horse sickness. The precise list of diseases depends on the country of origin and may vary with disease reports from that country. Prior to shipment into Canada, from a country other than the USA, horses will be subject to a period of quarantine in that country, the precise details of which will depend on the country of origin. Upon arrival in Canada, all horses from countries other than the USA are subject to 7 days quarantine with repeated health checks and a repeat of all relevant serology prior to release into the population. Horses may not be given a live vaccine (including influenza) within 14 days of travel to Canada.

#### ***Caribbean (T. King)***

Horses are imported directly into the Caribbean from the USA, Canada, France, UK and Ireland, whereas horses from other countries are imported via either the USA or Europe. No request for importation from Asia has been received. All horses imported from outside the region and those not imported for racing are quarantined in Barbados for a minimum of 10 days. All horses imported to Barbados for racing purposes from the Caribbean territories of Jamaica, Trinidad and Martinique are quarantined for a minimum of only 5 days. The horses must also be on an official vaccination programme and have received a booster dose between 7 and 30 days prior to entry to the island.

#### ***Argentina (E. Nosetto)***

The Ministry of Agriculture, Livestock and Fisheries establishes the animal sanitary rules in Argentina through the National Veterinary Service (SENASA). The rules relating to equine influenza were introduced in July 1987 and apply to all horses in the country. Working horses living on farms and not used for sporting or recreational purposes must be vaccinated annually in June. All horses used for sports such as racing, polo, pato,

jumping, riding and shows, all horses used to obtain biological derivatives, in livestock markets, for national defence and others must be vaccinated every 3 months (March, June, September and December). Horses being exported or imported must be vaccinated at least 15 days and not more than 60 days before departure. In all cases vaccination must be carried out with vaccines approved by SENASA and performed by an accredited veterinarian. Horses that do not have appropriate documentation of vaccination cannot be moved from one place to another.

#### ***Chile (M. Perez)***

Horses being imported into Chile must not have had clinical signs of influenza in the last 90 days and must have been vaccinated with a killed virus vaccine between 30 and 365 days before importation.

#### ***Mexico (J. Garcia Garcia)***

Importation of horses into Mexico requires a health certificate from an official veterinarian declaring freedom from communicable disease at an inspection conducted 16 days prior to entry. A vaccination certificate confirming vaccination against western and eastern equine encephalomyelitis 14 days previously is also required. Horses must not have been vaccinated against influenza within 40 days of importation and must have been officially declared negative for equine infectious anaemia and *Taylorella equigenitalis* infections.

#### ***United Arab Emirates (R. Wernery)***

Permission to permanently export horses to the UAE must be sought from the Animal Welfare Department of the Ministry of Agriculture and Fisheries (MAF/AWD) before the shipment leaves the exporting country. This permission is sought with the submission of a completed Import Permit Application to the MAF/AWD who may request copies of test results and proof of vaccinations. Depending on the health status of the exporting country, horses will be subjected to various blood tests with negative results required within 21 days of export. The tests must be conducted in an approved laboratory. All horses must be fully vaccinated against equine influenza. A health certificate issued by a veterinary surgeon approved by the authority of the exporting country to carry out export certification and endorsed by a

government veterinarian must accompany all horses sent to the UAE. Since an outbreak of influenza in the UAE in 1995, all imported horses are subject to a minimum of 6 days isolation in an officially approved isolation centre and will be subjected to further tests at the discretion of MAF. After 3 days in isolation, horses are screened for influenza by nasal swabs using the Directigen Flu-A immunoassay. In order to reduce the risk of importing an infected animal, all horses must have been vaccinated between 14 and 60 days prior to entry. This vaccination must comprise a completed primary course or a booster vaccination to a known history. All horses must arrive by air at one of the approved international airports at Dubai, Abu Dhabi or Sharjah, unless special authority is granted to land elsewhere.

#### *Australia (D. Powell)*

Pre-export requirements are that the premises from which horses are being exported should have been free from equine influenza for the past 3 months, that horses undergo 21 days of quarantine and must have been vaccinated against influenza within the previous 6 months. A veterinarian must certify horses following a health examination within 24 h

of export. Horses undergo 14 days of post export quarantine following importation to Australia. The Australian authorities have included equine influenza in their contingency plan for control of novel diseases (Disease Strategy Equine Influenza, AUSVETPLAN, 1996). AUSVETPLAN is a series of technical response plans that describe the proposed Australian approach to an exotic disease incursion. The documents provide guidance based on sound analysis, linking policy, strategies, implementation, co-ordination and emergency management plans.

#### *Japan (T. Matsumura)*

Racehorses attending international races in Japan must satisfy import regulations with respect to vaccination and freedom from influenza infection at the exporting premises in specified countries (UAE, Hong Kong, UK, France, Italy, Germany, Ireland, Australia, Canada and USA). Horses must be vaccinated within 12 months of export and have either received 2 injections of a primary course of vaccination or have received a booster injection not more than 12 months apart. Horses must be kept on premises that have not had equine influenza for at least 60 days prior to export.

## INADEQUATE MONITORING OF FIELD INFLUENZA AS A FACTOR CONTRIBUTING TO THE LACK OF CONTROL OF INFLUENZA

J. R. Newton

The following points were raised from the UK perspective as factors contributing to inadequate monitoring of field influenza outbreaks:

- Influenza is not a notifiable disease in the UK and as such there is no mandatory requirement to report it or have suspected outbreaks investigated.
- The high cost of virological investigations is frequently prohibitive. In order to overcome this, tests are frequently conducted free of charge by the Animal Health Trust.
- Mild clinical signs are often seen in vaccinated horses, which may be attributed to other infectious causes or even overlooked altogether.
- There is still a widespread reliance on non-specific blood tests (particularly haematology) for the diagnosis of viral infections in horses.
- There is a reliance on passive surveillance for the initial notification of disease outbreaks to the AHT. The Newmarket veterinary practices have been very important in this for many years.
- Influenza is often ignored as a differential diagnosis of respiratory disease in horses.
- The submission of acute nasopharyngeal swab samples continues to be limited. Some geographical differences occur; samples are more readily submitted from Newmarket than

from elsewhere in the UK. This probably reflects the proximity of the practices to the AHT as well as differences in the perceived benefit from obtaining a confirmed diagnosis by some veterinary surgeons.

- There may be limitations to the availability of resources for intensive and immediate investigations following the initial diagnosis of influenza. Resources may be stretched elsewhere as was the case with an outbreak of equine viral arteritis in 1993, and despite the equine industry subsidy of outbreak investigations covering laboratory costs, there is still a reliance on good will from practising veterinary surgeons.
- There is a difference in the needs of interested parties from surveillance. Veterinary surgeons

and horse owners generally only want the initial diagnosis, although they ultimately benefit from updated vaccines, whereas others such as researchers and vaccine manufacturers want detailed epidemiological information.

- The use of vaccination in the face of an influenza outbreak precludes maximal epidemiological benefit from serological data.
- Virus isolation is generally more difficult in vaccinated horses, which generally produce less virus than non-vaccinated horses that are infected. Furthermore, it has been more difficult to isolate influenza viruses by conventional egg inoculation in recent years MDCK cells were used to isolate viruses in the UK in 1998.

## DISCUSSION

**D. Powell:** I would like to emphasise the importance of the difference between permanent importation and the temporary movement of horses for competitions.

**P. Morley:** Regarding the imperfect sensitivity of the Directigen Flu-A assay, might this increase the risk of importing a clinically normal but potentially infectious horse?

**D. Powell:** The Directigen test is better than nothing and I feel reasonably confident with it as a diagnostic test and screening assay despite some limitations.

**P. Morley:** How many horses have been picked up as positive by the Directigen assay and have any been missed?

**T. Chambers:** I can present some data from around 200 samples we have tested using the Directigen assay. These data showed that the test had a positive predictive value (ie the likelihood that a horse with a positive result has influenza) of 80–85%, and a negative predictive value (ie the likelihood that a horse with a negative result does not have influenza) of 80–85%. In an outbreak in Hong Kong, investigated by Professor Ken Shortridge, there were more horses that tested

positive by Directigen assay than by virus isolation. I would question whether the method of swabbing was a significant factor.

**D. Powell:** I am not aware that any horses have tested positive during the quarantine programme since the last outbreak in Hong Kong.

**J. A. Mumford:** The biggest problem in the system of international movement is that importing countries rely on knowledge of infection in the exporting country but this is often not known and not investigated.

**D. Powell:** I agree that this is a major weakness, added to which it seems that, occasionally, health certificates are being signed inaccurately.

**J. A. Mumford:** There is obviously a need to improve the surveillance of equine influenza.

**T. Cordes:** As the first line of defence, there is a need to carry the message to veterinarians in the field.

**D. Wilson:** Declaring a premise free from disease is often extremely difficult for veterinarians, especially on large premises with many horses. I feel that there is every likelihood that infectious

disease present on these premises could be overlooked.

**L. Coffman:** There could be problems with lawsuits when vets asked to sign certificates are not able to do so. There is a need for qualifying statements on certificates along the lines that a general health certificate was signed to the best of their knowledge and applied at the time of that inspection.

**D. Powell:** The criteria for the optimal chance of isolating influenza virus are that the affected horses are part of a large group and that accurate records of the rectal temperatures of individual horses are kept for the preceding 48 h period. In my opinion it is only worth swabbing horses with rectal temperatures of  $\geq 102.5^{\circ}\text{F}$  ( $41.2^{\circ}\text{C}$ ) in the last 48 h.

**T. Chambers:** I think it is also worth swabbing the horses in the adjacent stalls to the pyrexial horses.

**R. Holland:** Frequently, we cannot isolate influenza virus if there has been a mucopurulent discharge of more than 2 days' duration, but the horses in the next-door stalls are positive. The problem of confidentiality does not really arise in my work in Kentucky yards, but there is a problem in that veterinarians often do not want to know that it is influenza because they have been vaccinating against it.

**L. Coffman:** It was pointed out that there has been very good correlation between virus isolation results and Directigen Flu-A test results performed on the same swabs. Therefore, it probably makes good sense for veterinarians to send Directigen test positive swabs only to virology laboratories for virus isolation in eggs. Subsequent biosecurity measures should be based on both clinical signs and Directigen testing.

**T. Chambers:** I agree, we generally only inoculate swab extracts from Directigen test positive swabs. The rapid result from the Directigen tests is of considerable benefit and can be obtained readily without the need for trained personnel or expensive equipment.

**L. Coffman:** There is a real need to recognise the practical issues related to outbreak investigations in that there is often a difference in the level of response according to the value of the animals involved. There are large numbers of pleasure horses in particular, which travel frequently and the monitoring of health by certification needs to be improved. There should be some provision of resources for this by the industry and a regulatory epidemiologist, for example, might be a good way forward.

**D. Wilson:** I think the immediacy of diagnosis is the most important factor in control and, if horses are Directigen test positive, there is no need to send a sample for virus isolation, this is only academic.

**A. Hay:** I do not see any conflict between the 2 diagnostic methods because virus isolation and characterisation of isolates leads to improved vaccines containing the most epidemiologically relevant strains.

**B. Hardy:** There may be legal issues for practitioners who recover viral isolates from horses under their care following vaccination with an 'out-dated' vaccine and which may have received greater protection from another available vaccine with more relevant strains.

**M. Barrandeguy:** My feeling is that owners and veterinary surgeons want to know about influenza and this generally leads to good co-operation.

**T. Chambers:** It takes time to get information about individual isolates back to veterinarians, and often they are not interested in detailed information about these viruses. They really only want the disease to be differentiated from equine herpesvirus and strangles, which are the main differential diagnoses. They also want to know what they could do to improve things in the future, particularly with respect to their choice of vaccines. Although the University of Kentucky do not charge for conducting virus isolation on swabs submitted from suspected outbreaks, vets still need some incentive to send these swabs and this sort of information is one way of motivating them.

# SESSION 7:

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# THE WAY AHEAD

*A series of parallel meetings on 'the role of vaccine manufacturers', 'international movement and disease control', and 'extending the surveillance network' were held in order to formulate the conclusions and recommendations relating to these areas arising from the meeting.*

## ROLE OF VACCINE MANUFACTURERS

Chairman: B. Hardy  
Rapporteur: T. Chambers

Manufacturers are moving towards updated strains but at the time of the meeting none was yet licensed which contained both updated Eurasian and American isolates (NB: *since the meeting, a number of vaccines containing viruses from both sublineages have become available*).

There is an urgent need to establish criteria upon which to base recommendations for a change of strain. Information should be communicated to manufacturers rapidly.

Further challenges in horses are needed to test validity of post infection ferret sera cross reactivity as a predictor of cross protection in horses.

A uniform challenge system for assessing vaccine efficacy should be established. Trials should be blinded. Minimum severity levels should be set and suitable challenge viruses agreed upon based on surveillance data Agreement on standardised methods of data analysis and methods of reporting antibody titre should be developed.

It was recognised that harmonisation of licensing and regulatory procedures is highly desirable. This should include facilitation of introducing appropriate overseas strains into

vaccines in the USA and shipment of isolates into the USA. It was recommended that an *ad hoc* group comprising the regulatory bodies represented at this meeting be established to facilitate discussion on harmonisation of licensing requirements. The OIE Standards Commission should be asked to endorse initiatives. VICH was identified as a possible vehicle through which this group could operate. Companies should be asked to identify the major discrepancies between the US and European regulations that require duplication of testing.

Vaccination schedules need to be rationalised as there is a significant period of susceptibility between the second and third vaccinations when the latter is given at 6 months.

A system of vaccination certificates is necessary, including horse identity, batch and vaccine name.

Interference of maternal antibody was considered to be an important issue in the USA but less so in Europe.

A protocol for investigation and management of disease outbreaks should be developed, together with a programme for clinicians in terms of education and communication.

## INTERNATIONAL MOVEMENT AND DISEASE CONTROL

Chairman: D. Powell  
Rapporteur: D. P. Lunn

Quarantine conditions need to be defined more precisely to eliminate the failures that have contributed in the past to the spread of equine influenza. Examples include the outbreaks in South Africa in 1986 and Hong Kong in 1992. The 'all in-all out' policy is critical to the success of influenza quarantine control. A period of 14 days is considered ideal for quarantine but may not always be practical so 7 days may have to be accepted. Specific biosecurity recommendations should be

developed for quarantine facilities. There are good models for import regulations relating to internationally competing horses in the United Arab Emirates, Hong Kong, Australia and Japan. These should be considered for adoption by other countries.

The impact of regionalisation, as exemplified by the European Community, on the spread of disease was recognised. Country boundaries may no longer be considered adequate to limit the

spread of disease. Therefore, it is often more relevant to adopt policies on a regional rather than a country level.

The import regulations mentioned above emphasise the importance of more extensive use of rapid testing procedures such as the Directigen Flu-A immunoassay. Rapid tests are generally under-used and their value not appreciated. Further assessment of rapid tests and education regarding their value could increase their use.

More extensive use of effective influenza vaccines would reduce the disease incidence and hence the risk associated with movement of horses. Antibody testing is valuable in assessing levels of vaccine-induced immunity. There is a need for a contingency plan for the possible emergence of a

new subtype. This should include, for example, the way in which such an event would impact on movement and control measures. The Australian vet plan (AUSVETPLAN) contains useful practical information on how to handle the introduction of an exotic disease.

Health certification is a key factor in disease control. Health certificates need to contain relevant and reliable information, which may be aided by electronic identification of animals.

Any recommendations must have practical acceptance in the horse industry and need to be based on proper risk assessment and economic analysis. There is a need for education as well as practical recommendations to get co-operation from the horse industry.

## **EXTENDING THE SURVEILLANCE NETWORK**

Chairman: J. A. Mumford

Rapporteur: H. Townsend

A surveillance system is vital for the detection and identification of new and emerging strains of equine influenza and for understanding the epidemiology. It also enables the economic impact of the disease to be assessed and can support clinical management and control of influenza. It was agreed that surveillance should be a global effort to reflect international movement of horses.

The recommendation from the last meeting of WHO/OIE experts that a rapid communication system be established has not yet been initiated. A communication system and format to relay outbreak information and identification of new isolates is recommended. A website set up by AHT was proposed to increase interest, education and awareness of surveillance efforts which would act in concert with the International Collating Centre.

In addition, an e-mail list server for rapid dissemination of information to interested individuals should be established. The key role of the veterinary profession in surveillance was recognised and a model surveillance programme would be drafted to facilitate better diagnosis, to promote the submission of viruses to OIE Reference Laboratories and which ensured rapid results to the submitting veterinary surgeons. Advice would be sought from directors of

diagnostic laboratories and from equine associations.

It was recognised that success in isolating new viruses and collecting epidemiological data is dependent on co-operation of horsemen, veterinary practitioners, local laboratories, Departments of Agriculture and OIE reference laboratories. In order to encourage cooperation of the horse industry, a public relations programme should be developed to communicate our aims, needs and benefits to the horse industry through publications such as diagnostic laboratory newsletters, equine practitioner newsletters (eg AAEP, BEVA), and equine publications (eg *The Horse*).

The anticipated protocol would be submission of samples to regional laboratories for initial processing which would at least include the use of capture ELISA tests and perhaps attempts to isolate the virus. Positive samples would be forwarded to reference OIE or WHO laboratories for virus isolation and/or virus characterisation and it was recognised that the importance of making virus isolation attempts needed to be publicised.

It was recommended that serological techniques should be better standardised with the provision of reference sera and inter-laboratory comparisons conducted to establish acceptable test protocols.

In order to encourage sample submission, the following ideas were suggested:

- make all interested parties aware of the importance of the effort;
- advise practitioners of their obligation to report vaccine breakdowns;
- ensure rapid reporting of results to practitioners;
- educate practitioners about rapid test technology and make them aware that the diagnosis of influenza can be confirmed in a matter of hours;
- communicate information on vaccine efficacy and vaccination protocols;
- provide protocols for managing outbreaks of infectious respiratory disease;
- present summary results at meetings of local veterinary associations;
- encourage practitioners to contact their local laboratory as soon as they suspect an outbreak of influenza.

It was agreed that a list of collaborators be developed through regional laboratories and referral centres such as the colleges of veterinary medicine and presentations given to the national equine veterinary associations, particularly AAEP.

In order to obtain the cooperation and feedback from the regional laboratories in North America it was recommended that a presentation be given at their association meeting.

The OIE and other specialist laboratories should institute a training programme to ensure that local laboratories are able to perform virus detection and isolation techniques. Training should also be promoted internationally and networks developed in other regions such as South America. Practitioners should be advised to report vaccine failures as well as other adverse reactions to vaccine manufacturers and licensing authorities. The implications associated with reporting a vaccine failure highlights the need for an accurate diagnostic system on an international scale. Vaccine companies may be willing to assist with this process as part of product support and facilitate the diagnostic work.

## MAIN CONCLUSIONS AND RECOMMENDATIONS OF THE MEETING

- It is important to increase the rate of diagnosis and broaden the geographical areas covered by equine influenza surveillance. To achieve this, veterinarians should be encouraged to participate by emphasising the clinical benefits that can be obtained by rapid and accurate diagnosis and implementation of appropriate management regimes.
- Steps need to be taken to improve the collation and dissemination of surveillance information amongst the OIE/WHO panel (eg via e-mail or web-based information services).
- Surveillance should be extended to provide a sound basis for recommendations to change vaccine strains.
- The criteria on which decisions to change a strain are made should be supported by further experimental data and decisions should be communicated rapidly.
- Groups carrying out challenge studies should form a working party to recommend minimum uniform standards for challenge systems.
- A small animal model should be developed for assessing the impact of antigenic drift on equine influenza vaccine efficacy to be used in parallel with data arising from antigenic analysis with ferret sera.
- Epidemiologists should aim to obtain more field data in order to assess vaccines in terms of potency, appropriate strains and the vaccination schedules with particular reference to the interfering role of maternally-derived antibody and the timing of the third vaccination
- Spread of infection in vaccinated horses is an important criterion. Collection of these data is dependent on the cooperation of the veterinary

- profession, ie collection of samples for specific diagnosis and vaccination histories.
- Development of a standard form for reporting outbreaks would be valuable. For this an identification system is required, which is a major hurdle.
- Manufacturers should be encouraged to switch to SRD for in-process potency testing and SRH for antibody measurement (because of the reliability and reproducibility of these methods) now that reference preparations are available. In this context more data should be

## RECOMMENDATIONS FOR VIRUS VACCINE STRAINS

### *H7N7 (equi-1) subtype*

Vaccine manufacturers may wish to retain an H7N7 component in their products, but it was agreed that there was no epidemiological evidence to support the need to maintain H7N7 viruses in current vaccines. Isolation of H7N7 virus has not been confirmed since before 1980.

### *H3N8 (equi-2) subtype*

There was no evidence that Miami/63-like viruses

were circulating. This virus should be removed from vaccines as original antigenic sin may suppress the specific antibody response to more up-to-date viruses.

There is evidence that the American-like and European-like viruses are sufficiently different to compromise vaccine efficacy. It is recommended, therefore, that representatives of both subfamilies should be included (eg Kentucky/94 or Newmarket/1/93 as American-like viruses, and Newmarket/2/93, Borlänge/91 or Suffolk/89 as



# INTERNATIONAL EFFORT TO IMPROVE CONTROL OF INFLUENZA: REFERENCES AND REAGENTS

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## STEPS TO IMPROVE POTENCY AND EFFICACY OF INFLUENZA VACCINES

### *In vitro test (Single Radial Diffusion) validated as method to measure active antigenic content of vaccine*

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### *Standard reagents prepared for single radial diffusion*

Antisera against HA of:

- Prague/56 (H7N7)
- Miami/63 (H3N8)
- Kentucky/81 (H3N8)
- Newmarket/1/93 (H3N8) (Am)
- Newmarket/2/93 (H3N8) (Eur)

### *Reproducibility of HI and SRH tests for measuring antibody to HA*

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Viruses:

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- Newmarket/1/93 (H3N8) (American)
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