2010

Seroprevalence of equine influenza virus in northeast and southern Mexico

B. J. Blitvitch
Iowa State University, blitvich@iastate.edu

L. A. Ibarra-Juarez
Universidad Autonoma de Nuevo Leon

A. J. Cortes-Guzman
Secretaria de Salubridad y Asistencia, Chilpancingo, Guerrero, Mexico

J. J. Root
USDA/APHIS/WS National Wildlife Research Center, jeff.root@aphis.usda.gov

A. B. Franklin
USDA National Wildlife Research Center, alan.b.franklin@aphis.usda.gov

See next page for additional authors

Short Communications

Seroprevalence of equine influenza virus in north-east and southern Mexico

B. J. Blitvich, L. A. Ibarra-Juarez, A. J. Cortes-Guzman, J. J. Root, A. B. Franklin, H. J. Sullivan, I. Fernandez-Salas

EQUINE influenza A virus (EIV) is a highly infectious respiratory pathogen of horses (Hannant and Mumford 1996, Palese and Shaw 2007). The illness is characterised by an abrupt onset of fever, depression, coughing and nasal discharge, and is often complicated by secondary bacterial infections that can lead to pneumonia and death. Two subtypes of EIV, H3N8 and H7N7, have been isolated. The H7N7 subtype was first isolated from a horse in Czechoslovakia in 1956 (Prague/56), and the H3N8 subtype was first isolated from a horse in Miami in 1963 (Sovinova and others 1958, Waddell and others 1963). The last confirmed outbreak of H7N7 occurred in 1979, and this subtype is now considered to be either extinct or circulating at low levels in a few geographical areas (Ismail and others 1990, Webster 1993, Singh 1994, Madic and others 1996, van Maanen and Cullinane 2002). The H3N8 subtype is a common cause of disease in horses worldwide, particularly in areas where vaccination is not routinely performed (Paillot and others 2006).

Phylogenetically, the H3N8 subtype can be separated into five distinct clades, denoted as predivergence, Eurasian, American (Kentucky), Florida clade 1 and Florida clade 2 (Bryant and others 2009). The predivergence clade is composed of isolates from the 1960s to 1980s that are now extinct in nature. The Eurasian and American lineages emerged in the 1980s and continue to circulate (Daly and others 1996). These lineages were initially named on the basis of the geographical locations from which they were isolated, although strains in the American lineage have since been isolated in Europe. Evolution of the American lineage has resulted in the emergence of American (Kentucky), Florida clade 1 and Florida clade 2 (Bryant and others 2009).

There is little information on the seroprevalence and geographical distribution of EIV in Mexico. Previous studies of the prevalence and clinical manifestations associated with influenza virus infections in Mexico have generally focused on human beings and birds (Ayora-Talavera and others 2005, Cabello and others 2006, Kuri-Morales and others 2006, Villarreal 2006, Senne 2007). The overall aim of the present study was to estimate the seroprevalence of EIV in two geographically distinct areas of Mexico, Nuevo Leon State in north-east Mexico and Guerrero State in the south of the country.

A total of 242 horses at 10 study sites (114 horses from three sites in Nuevo Leon State and 128 horses from seven sites in Guerrero State) were sampled between September 2007 and May 2008 (Table 1). All of the study sites were on privately owned ranches or farms. According to the owners, none of the horses had a history of travel and none had been vaccinated against EIV. All the horses appeared healthy at the time of sampling.

Serum samples were tested for antibodies to EIV by epitope-blocking ELISA (bELISA) (Sullivan and others 2009). This assay utilises the influenza A virus nucleoprotein-specific monoclonal antibody clone A1 (Millipore) and recombinant influenza A virus nucleoprotein (Imgenex). The influenza A virus nucleoprotein is well conserved (Gorman and others 1990), and because of this the bELISA can detect antibodies to all influenza A virus subtypes including H3N8 and H7N7. A subset of bELISA-positive sera was further analysed by haemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests, which were initially performed at the National Veterinary Service Laboratories (NVSL) in Ames, Iowa, USA. The HI tests were done using the influenza A/equine/Kentucky/1/81 (H3N8), A/equine/Miami/1/63 (H3N8), and A/equine/Prague/1/56 (H7N7) reference strains. The NI tests were done using standard N1-N7 and N9 reference reagents, and N8 equine/Miami/63 reference reagent. Additional HI testing was then performed at the Gluck Equine Research Center (GERC) at the University of Kentucky in Lexington, Kentucky, USA. This analysis was done using eight strains of H3N8 that represent all five clades (Table 2), to provide information on the lineage and origin of the EIV strain(s) circulating in Mexico.

Overall, 94 (39 per cent) horses had antibodies to EIV by bELISA (Table 1). The seroprevalence for EIV in the horses in Guerrero State was 22 per cent, and among the horses in Nuevo Leon State it was 58 per cent. Sera from 10 horses (five in Nuevo Leon State and five in Guerrero State) that were positive by bELISA were further examined by HI and NI tests at the NVSL. All 10 samples had antibodies to the H3N8 subtype, and none had antibodies to the H7N7 subtype. The same 10 samples were then submitted to the GERC and analysed by the HI test using a broad panel of H3N8 strains. For each sample, the HI titre was highest when A/equine/Kentucky/1/97 (H3N8), which belongs to the American (Kentucky) clade, was used (Table 2); the HI titres ranged from 40 to 640. In seven samples, the HI titres were equally high when A/equine/Ohio/1/2003 (H3N8) was used. This strain belongs to Florida clade 1. Interestingly, on two occasions, the HI titres were equally high when A/equine/Aboyne/1/05 (H3N8), a recent member of the Eurasian clade, was used.

Veterinary Record (2010) 166, 565-566 doi: 10.1136/vr.b4845

B. J. Blitvich, PhD,
Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA
L. A. Ibarra-Juarez, MSc,
I. Fernandez-Salas, PhD,
Laboratorio de Entomologia Medica, Facultad de Ciencias Biologicas, Universidad Autonoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon 66450, Mexico
A. J. Cortes-Guzman, MSc,
Departamento de Control de Vectores, Secretaria de Salubridad y Asistencia, Chilpancingo, Guerrero 39000, Mexico
J. J. Root, PhD,
A. B. Franklin, PhD,
H. J. Sullivan, MSc,
Wildlife Services, United States Department of Agriculture, National Wildlife Research Center, Fort Collins, CO 80521, USA

E-mail for correspondence:
blitvich@iastate.edu

Provenance: not commissioned; externally peer reviewed

TABLE 1: Seroprevalence for equine influenza virus in horses sampled at 10 study sites in north-east and southern Mexico

Study site	State	Number of horses sampled	Number (%) seropositive
Cadereyta Lienzo Charro	Nuevo Leon	56	33 (58.9)
La Estanzuela	Nuevo Leon	18	12 (66.7)
San Bernabé	Nuevo Leon	40	21 (52.5)
Acapulco	Guerrero	33	1 (3.0)
Charco La Puerta	Guerrero	35	1 (2.9)
El Tamarindo	Guerrero	1	0
Las Iguanas	Guerrero	21	2 (9.5)
Playa Linda	Guerrero	5	1 (20.0)
San José Ixtapa	Guerrero	8	4 (50.0)
Santiago	Guerrero	25	19 (76.0)
Total		242	94 (38.8)

TABLE 2: Serological data for horses analysed by the haemagglutination inhibition test using multiple influenza virus H3N8 strains

Horse number	Study site	State	Influenza virus strain							
			Miami/63*	KY/81*	Aboyne/05†	Nmark/93†	KY/97‡	KY/98‡	OH/03§	Rich/07¶
H-5	La Estanzuela	Nuevo Leon	<10	<10	10	<10	80	<10	20	10
H-21	San Bernabé	Nuevo Leon	<10	40	80	<10	160	40	160	40
H-22	San Bernabé	Nuevo Leon	<10	80	160	<10	160	40	160	80
H-37	San Bernabé	Nuevo Leon	<10	10	80	<10	80	10	80	40
H-41	San Bernabé	Nuevo Leon	<10	40	40	<10	80	20	80	40
H-63	Santiago	Guerrero	20	40	80	10	160	20	160	40
H-72	Santiago	Guerrero	20	40	160	20	320	20	320	80
H-83	Santiago	Guerrero	10	40	160	20	320	40	320	40
H-84	Santiago	Guerrero	20	160	320	20	640	160	320	160
H-187	Las Iguanas	Guerrero	<10	<10	10	<10	40	<10	<10	<10

* Predivergence
† Eurasian
‡ American (Kentucky)
§ Florida clade 1
¶ Florida clade 2
Miami/63 A/equine/Miami/1/63 (H3N8), KY/81 A/equine/Kentucky/2/81 (H3N8), Aboyne/05 A/equine/Aboyne/1/05 (H3N8), Nmark/93 A/equine/Newmarket/2/93 (H3N8), KY/97 A/equine/Kentucky/1/97 (H3N8), KY/98 A/equine/Kentucky/1/98 (H3N8), OH/03 A/equine/Ohio/1/2003 (H3N8), Rich/07 A/equine/Richmond/1/07 (H3N8)

Of the study sites at which over 20 horses were sampled, the rate of seropositivity for EIV ranged from approximately 52 to 59 per cent in Nuevo Leon State, and 3 to 76 per cent in Guerrero State (Table 1). The youngest seropositive horse in Nuevo Leon State was a 12-month-old stallion sampled in September 2007, suggesting that the most recent EIV infection in the region had occurred during or after 2006. Another three seropositive horses in Nuevo Leon State were two years old or younger. In Guerrero State, the youngest seropositive horse was a three-year-old stallion sampled in April 2007, suggesting that the most recent EIV infection in this region had occurred during or after 2004.

In summary, a moderate seroprevalence for EIV was detected in horses in southern Mexico, and a high seroprevalence was detected in horses in the north-east of the country. The high HI antibody titres to Kentucky/97 (Florida clade 1) and Ohio/03 (American [Kentucky]) and low titres to Richmond/07 (Florida clade 2) suggest that the predominant EIV strains in Mexico belong to Florida clade 1 and/or the American (Kentucky) clade. These findings tend to agree with those of Bryant and others (2009) who provided evidence that the predominant EIV isolates in North America and Europe from 2006 to 2007 belong to Florida clades 1 and 2, respectively. Recent work by the authors' laboratory has also shown that there is a moderately high seroprevalence (25 per cent) for EIV in unvaccinated horses in the Yucatan Peninsula of Mexico (Loroño-Pino and others 2010). Taken together, these findings indicate that EIV is a common cause of infection in horses in Mexico, and that continued surveillance for EIV in Mexico is warranted.

Acknowledgements

This study was supported by a cooperative agreement with the United States Department of Agriculture (reference number 0874880712CA) and start-up funds were provided by Iowa State University.

References

AYORA-TALAVERA, G., CADAVIECO-BURGOS, J. M. & CANUL-ARMAS, A. B. (2005) Serologic evidence of human and swine influenza in Mayan persons. *Emerging Infectious Diseases* **11,** 158-161

BRYANT, N. A., RASH, A. S., RUSSELL, C. A., ROSS, J., COOKE, A., BOWMAN, S. & OTHERS (2009) Antigenic and genetic variations in European and North American equine influenza virus strains (H3N8) isolated from 2006 to 2007. *Veterinary Microbiology* **138,** 41-52

CABELLO, C., MANJARREZ, M. E., OLVERA, R., VILLALBA, J., VALLE, L. & PARAMO, I. (2006) Frequency of viruses associated with acute respiratory infections in children younger than five years of age at a locality of Mexico City. *Memórias Do Instituto Oswaldo Cruz* **101,** 21-24

DALY, J. M., LAI, A. C., BINNS, M. M., CHAMBERS, T. M., BARRANDEGUY, M. & MUMFORD, J. A. (1996) Antigenic and genetic evolution of equine H3N8 influenza A viruses. *Journal of General Virology* **77 (Pt 4),** 661-671

GORMAN, O. T., BEAN, W. J., KAWAOKA, Y. & WEBSTER, R. G. (1990) Evolution of the nucleoprotein gene of influenza A virus. *Journal of Virology* **64,** 1487-1497

HANNANT, D. & MUMFORD, J. A. (1996) Equine influenza. In Virus Infections of Equines. Eds M. J. Studdert, M. C. Horzinek. Elsevier Science. pp 285-293

ISMAIL, T. M., SAMI, A. M., YOUSSEF, H. M. & ABOU ZAID, A. A. (1990) An outbreak of equine influenza type 1 in Egypt in 1989. *Veterinary Medical Journal Giza* **38,** 195-206

KURI-MORALES, P., GALVÁN, F., CRAVIOTO, P., ZÁRRAGA ROSAS, L. A. & TAPIA-CONYER, R. (2006) Mortality due to influenza and pneumonia in Mexico between 1990 and 2005. *Salud Pública De México* **48,** 379-384 (In Spanish)

LOROÑO-PINO, M. A., FARFAN-ALE, J. A., GARCIA-REJON, J. E., LIN, M., ROSADO-PAREDES, E., PUERTO, F. I., BATES, A., ROOT, J. J., FRANKLIN, A. B., SULLIVAN, H. J. & BLITVICH, B. J. (2010) Antibodies to influenza and West Nile viruses in horses in Mexico. *Veterinary Record* **166,** 22-24

MADIC, J., MARTINOVIC, S., NAGLIC, T., HAJSIG, D. & CVETNIC, S. (1996) Serological evidence for the presence of A/equine-1 influenza virus in unvaccinated horses in Croatia. *Veterinary Record* **138,** 68

PAILLOT, R., HANNANT, D., KYDD, J. H. & DALY, J. M. (2006) Vaccination against equine influenza: quid novi? *Vaccine* **24,** 4047-4061

PALESE, P. & SHAW, M. L. (2007) Orthomyxoviridae: the virus and their replication. In Fields Virology. Eds B. N. Fields, D. M. Knipe, P. M. Howley. Lippincott-Raven. pp 1647-1689

SENNE, D. A. (2007) Avian influenza in North and South America, 2002-2005. *Avian Diseases* **51**(1 Suppl), 167-173

SINGH, G. (1994) Characterization of A/eq-1 virus isolated during the equine influenza epidemic in India. *Acta Virologica* **38,** 25-26

SOVINOVA, O., TUMOVA, B., POUSKA, F. & NEMEC, J. (1958) Isolation of a virus causing respiratory disease in horses. *Acta Virologica* **2,** 52-61

SULLIVAN, H. J., BLITVICH, B. J., VANDALEN, K., BENTLER, K. T., FRANKLIN, A. B. & ROOT, J. J. (2009) Evaluation of an epitope-blocking enzyme-linked immunosorbent assay for the detection of antibodies to influenza A virus in domestic and wild avian and mammalian species. *Journal of Virological Methods* **161,** 141-146

VAN MAANEN, C. & CULLINANE, A. (2002) Equine influenza virus infections: an update. *Veterinary Quarterly* **24,** 79-94

VILLARREAL, C. L. (2006) Control and eradication strategies of avian influenza in Mexico. *Developments in Biologicals* **124,** 125-126

WADDELL, G. H., TEIGLAND, M. B. & SIGEL, M. M. (1963) A new influenza virus associated with equine respiratory disease. *Journal of the American Veterinary Medical Association* **143,** 587-590

WEBSTER, R. G. (1993) Are equine 1 influenza viruses still present in horses? *Equine Veterinary Journal* **25,** 537-538