

Report of 2018 equine influenza outbreak in Chile

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ABSTRACT. The present study reports an outbreak of equine influenza (EI) occurred in Chile in January 2018, with cases spread along the country, from the Atacama to the Magallanes Region. The virus identified corresponded to the H3N8 subtype, affecting equines, donkeys, and mules. Clinical signs ranged from mild to severe, with a higher mortality rate in donkeys.

Key words: equine influenza, outbreak, equines, donkeys, Chile.

INTRODUCTION

Since 1963 the equine influenza virus (EIV) H3N8 subtype has been the cause of numerous outbreaks of respiratory disease in horses worldwide, including South American countries where the horse industry is of major importance (Olguin Perglione *et al* 2016). Recognised as a common malady of equids for centuries, influenza is a highly contagious viral infection mainly characterised by fever, depression, a mucoid or mucopurulent discharge, coughing and impairment of normal behaviour (Timoney 1996). These signs usually become more evident between 1 and 2 days post-infection and can last several days (Glass *et al* 2002). The incubation period fluctuates depending on the preexisting immunity of the horse and the magnitude of the virus attack, but it can be as short as 24 hours (Cullinane and Newton 2013).

The EIV of the H3N8 subtype were initially assigned to a single cluster that evolved into two sublineages, American and Eurasian, according to the geographic region. The American lineage was divided into South American, Kentucky, and Florida sublineages. The strains of the Florida sublineage had amino acid mutations in the hemagglutinin (HA1) subunit, and this lineage diverged into Florida Clade 1 (FC1) and Florida Clade 2 (FC2), represented by A/equine/South Africa/4/2003 and A/equine/Richmond/1/07, respectively. The lineages FC1 and FC2 have been identified in outbreaks worldwide and are predominantly circulating EIVs (Favaro *et al* 2018).

In Chile, the first outbreak of equine influenza (EI) was described in 1963 (Fuschlocher *et al* 1963). In 1977, the EIV was isolated, corresponding to the H7N7 subtype (Casanova *et al.* 1977), later in 1985, the H3N8 subtype was isolated (Berríos *et al* 1986). Another outbreak was

reported in Chile in March 1992 where the isolate was identified as H3N8 subtype (Celedón *et al* 1992). In July 2006, horses from various regions of Chile presented fever, serous nasal discharge, dry cough, anorexia, and depression. The virus was identified as EIV H3N8 subtype (Müller *et al* 2009). During 2012, an extensive outbreak of EI (H3N8 subtype) occurred across multiple countries in South America. The epidemic was first reported in Chile, then spread to Brazil, Uruguay, and Argentina, where both vaccinated and unvaccinated animals were affected. The equine influenza epidemic in South America in 2012 was caused by a virus belonging to Florida Clade 1, similar to those circulating in the USA in 2011 (Beuttemüller *et al* 2016).

The herein presented report aims to describe the 2018 EI outbreak in Chile, and to further characterise the virus subtype and how it spread throughout the country.

MATERIAL AND METHODS

Two hundred and ninety nasopharyngeal swabs were collected between January 10th and March 20th 2018, from horses showing typical influenza clinical signs, including nasal discharge, cough, and pyrexia. Samples were collected and processed for diagnostic testing at SAG's Lo Aguirre laboratory (Official Veterinary Service of Chile - SAG). Nasopharyngeal swabs in brain-heart infusion or UTM (Universal Transport Medium) transport mediums were processed for molecular analysis of equine influenza virus. From a 50 µl aliquot of swab medium, viral RNA was extracted with the automated magnetic-bead-based MagMAX™-96 Viral Isolation kit (Applied Biosystems, Cat. AM1836-5, Foster City, CA, USA) according to manufacturer's instructions, and using the KingFisher™ Duo Prime Purification System (Thermo Scientific). The extracted RNA was processed by real-time qRT-PCR with primers and probes to detect the matrix gene (Torchetti 2016) with a CT value of 36 (cycle threshold cut off). The primer sequences are detailed in table 1.

Two positive samples were isolated in 10-day-old embryo specific pathogen free (SPF) chicken eggs. Three to five days after isolation, allantoic fluid was collected

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and tested for haemagglutination activity (OIE 2017). All samples were put together into one batch of eggs. An aliquot of each isolate was then recovered and processed by gel-based RT-PCR to detect the hemagglutinin gene with specific primers for H3 and H7, which have amplification products of 1700 and 1800 bp respectively (Müller *et al* 2005). The primer sequences are detailed in table 2.

The obtained HA nucleotide sequences (A/equine/Chile/2018) were edited with the BioEdit software v7.2.5. and aligned using ClustalW. The alignment used 43 reference HA sequences, including North-American, European and Argentinian isolates, as well as vaccine isolates available in Genbank. For comparative purposes, the analysis was performed with a partial HA1 sequence gene (930 bp). The phylogenetic tree was created by the maximum likelihood (ML) method using the MEGA6 software. The statistical validity of the sequence comparisons was tested by bootstrap analysis using 1,000 bootstrapped data sets.

RESULTS AND DISCUSSION

In January 2018, it was reported to SAG that pure breed Chilean horses from Colina (Metropolitan Region) were experiencing respiratory signs compatible with equine influenza virus infection. On January 11th, 2018 the affected farm was visited and samples from 3 horses were collected and submitted for analysis. The presence of EIV was confirmed by SAG Lo Aguirre’s laboratory using RT-PCR technique (Muller *et al* 2019, Torchetti 2016). To verify the antigenic response, neuroaminidase and hemagglutinin genes were sequenced. The nucleotide sequence of hemagglutinin was submitted to GenBank (MH824795).

Table 1. Primers and probes for the detection of the matrix gene of EIV (Torchetti 2016).

Name	5'- 3' Sequence
Primer M +25	AGATGAGTCTTCTAACCGAGGTCG
Probe M +64	FAM- TCAGGCCCCCTCAAAGCCGA-TAMRA
Primer M-124 2002	TGCAAAAACATCTTCAAGTCTCTG
Primer M-124 2009	TGCAAAGACACTTCCAGTCTCTG

Table 2. Forward and reverse primers for the detection of the hemagglutinin gene H3 and H7 subtypes. (Muller *et al* 2009).

Name	5'- 3' Sequence
Primer H3-F	ATGAAGACAACCATTATTTTGATACTACTG
Primer H3-R	TCAAATGCAAATGTTGCATCTGATGTTGCC
Primer H7-F	ATGAACACTCAAATTCTAATATTAGCCAC
Primer H7-R	TCATATACAAATAGTGCACCGCATGTTTC

The samples 15646-24 and 15781-3 were positive in the qRT-PCR targeting the matrix gene (CT values: 19.64 and 20.42 respectively). A high percentage of similarity was obtained with virus H3N8 from different geographic areas. Specific primers for N8 were used to sequence the neuraminidase gene from the samples 15989-1 and 16186 (Killian 2013), obtaining fragments with amplification products of 1450 bp. For the interpretation of the sequence obtained, it is important to understand that since 1987, H3N8 EIV genetic evolution originated two lineages: European (Sussex 89) and American lineage (Kentucky 94). Later, American lineage originated Florida lineage, which split into two clades: Florida 1 and Florida 2 (Daly *et al* 2011).

The phylogenetic tree includes all lineages mentioned above (figure 1). The Genbank accession numbers of the sequences used to create the tree are detailed in table 3. The recent Chilean isolate belongs to Clade 1, Florida lineage. North-American isolates described since 2013 belongs to this clade as well, with EIVs from outbreaks in South Africa and Australia (Daly *et al* 2011).

After the initial report received by the SAG, strict monitoring was performed and nasopharyngeal swab samples from suspected cases from the Atacama to the Magallanes region were collected and analysed (table 4). Results showed that 242/290 (83.4%) of the analysed samples were positive in the Real time RT PCR type A influenza matrix gene. From January 10th to March 20th, the SAG identified 255 EI affected premises between the regions of Atacama and Magallanes, involving a total of 9,296 susceptible equidae (table 5). The presence of this disease was communicated nationwide to all SAG personnel and to the association of equine veterinary specialists. The SAG also informed private veterinarians about the outbreak to encourage EI vaccination and to stop transport of the affected animals to other sites for at least the following 21 days.

Equine influenza is a disease of mandatory reporting to SAG and international notification to the World Organisation for Animal Health (OIE). In Chile, outbreaks of the disease have occurred sporadically, for example in 2006 (Müller *et al* 2009) and 2012 (Beuttemüller *et al* 2016) due to immune breaks caused by the discontinuation of vaccination programs. The occurrence of this endemic disease after several years could be due to poor herd immunity, caused by a decrease in the protective immunity that the vaccine confers, or it could be due to a lower application frequency of specific vaccines, particularly in the stratum equines of Chilean thoroughbred. Vaccination is not mandatory for horses; however, there are only an average of 58,000 doses available per year for immediate use. According to official data, and considering an estimated population of 300,000 equines, 20% of Chilean equines have been annually vaccinated for the past five years.

During this outbreak, the equines did not present substantial changes in the pattern of symptoms, maintaining a mild to severe condition, with cough being one of the main signs in affected horses. The disease was confirmed in a

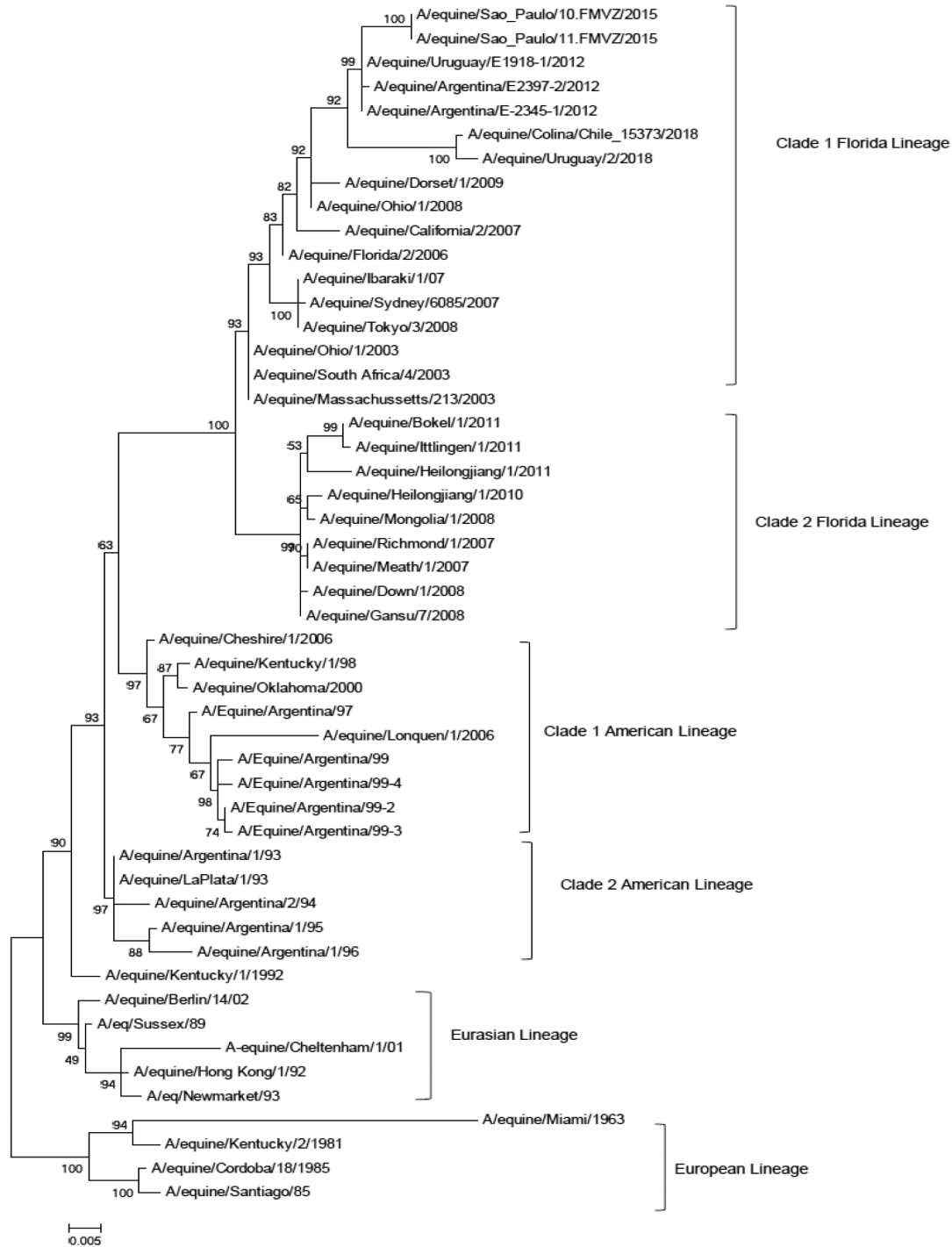


Figure 1. Phylogenetic tree created from nucleotide sequences of the HA1 portion of the haemagglutinin molecule of equine influenza H3N8 strain recently isolated in Lo Aguirre and prototype strains of the different lineages and clades. The tree was constructed using maximum likelihood (ML) method. Genbank accession numbers are available in table 3.

Thoroughbred and a Chilean pure breed horse (Caballo corralero).

In domestic and feral donkeys, the observed symptoms were respiratory distress, mucous purulent nasal secretion, and cough. Complicated cases presented mild respiratory signs, with cough, mucus, and fever. Pneumonia (pneumonic

foci in the apical, iliac and ventral mediastinal regions) and death were reported in 3/10 of those affected donkeys, most likely associated with secondary infection with bacterial agents, such as *Streptococcus equi*.

It is estimated that EIV has been circulating since December 2017 where it spread through equestrian sporting

Table 3. Sequences used in the phylogenetic analysis and their genbank accession numbers.

Name	Genbank accession N°
A/equine/Dorset/1/2009	CY054287.1
A/equine/Ohio/1/2008	GU045283.1
A/equine/California/2/2007	FJ195402.1
A/equine/Florida/2/2006	FJ195403.1
A/equine/Ibaraki/1/07	AB360549
A/equine/Sydney/6085/2007	GU045763
A/equine/Tokyo/3/2008	AB435160.1
A/equine/South Africa/4/2003	GU447312
A/equine/Ohio/1/2003	DQ124192
A/equine/Massachusetts/213/2003	DQ124193.1
A/equine/Heilongjiang/1/2010	JQ265982.2
A/equine/Mongolia/1/2008	AB436910.1
A/equine/Gansu/7/2008	EU794495.1
A/equine/Bokel/1/2011	CY100070.1
A/equine/Ittlingen/1/2011	CY107012.1
A/equine/Heilongjiang/1/2011	JQ265983.1
A/equine/Down/1/2008	JN222937
A/equine/Richmond/1/2007	FJ195395
A/equine/Meath/1/2007	JN222935
A/equine/Cheshire/1/2006	FJ195401.1
A/equine/Kentucky/1/98	AF197241
A/equine/Oklahoma/2000	AY273168.1
A/equine/Argentina/97	AY048077
A/equine/Lonquen/1/2006	EU926631
A/equine/Argentina/99	AY048081
A/equine/Argentina/99-4	AY048080
A/equine/Argentina/99-2	AY048078
A/equine/Argentina/99-3	AY048079
A/equine/Argentina/1/95	AF197244
A/equine/Argentina/1/96	AF197246
A/equine/Argentina/2/94	AF197245
A/equine/Argentina/1/93	L39913
A/equine/LaPlata/1/93	D30686
A/equine/Kentucky/1/1992	CY030149
A/equine/Berlin/14/02	EF541430.1
A/eq/Sussex/89	X85090.1
A/equine/Argentina/E-2345-1/2012	AHJ57548
A/equine/Argentina/E2397-2/2012	AOS87378
A/equine/Sao_Paulo/10.FMVZ/2015	AOX49483
A/equine/Sao_Paulo/11.FMVZ/2015	AOX49484
A/equine/Uruguay/E1918-1/2012	AOS87385
A/equine/Uruguay/2/2018	MH673717
A/equine/Hong Kong/1/92	L27597
A/eq/Newmarket/93	X85089
A/equine/Cheltenham/1/01	EF541435.1
A/equine/Kentucky/2/1981	CY028820
A/equine/Cordoba/18/1985	CY032301.1
A/equine/Santiago/85	AY383755.1

Table 4. Nasopharyngeal swabs collected by region.

Regions	Positive	Negative	Total
Atacama	3	0	3
Coquimbo	8	2	10
Valparaíso	37	14	51
Metropolitana	34	9	43
O'Higgins	9	2	11
Maule	33	2	35
Biobío	23	2	25
Araucanía	4	2	6
Los Ríos	3	0	3
Los Lagos	12	5	17
Aysén	69	5	74
Magallanes	7	5	12
	242	48	290

Table 5. Premises and horses affected by regions.

Regions	Premises affected	Susceptible horses	Cases
Atacama	27	784	161
Coquimbo	9	249	101
Valparaiso	32	709	186
Metropolitana	28	4,669	273
O'Higgins	11	127	50
Maule	44	603	264
Biobío	34	843	170
Araucanía	12	139	62
Los Ríos	3	247	105
Los Lagos	14	475	107
Aysén	36	353	165
Magallanes	5	98	57
	255	9,296	1,701

events, which involved travelling of affected horses to various parts of the territory, thus explaining the rapid spread to all equids that had not been properly immunised.

The spatial pattern of the cases, indicated a distribution throughout the country from the Atacama to the Magallanes Region. The report of cases compatible with EI were notified and recorded. To characterise the real distribution and dissemination of the virus in the national equine population, samples were collected for EI molecular diagnosis. Cases were confirmed in donkeys from the Valparaíso and Atacama regions.

The north region of the country (Arica and Parinacota, Tarapacá, and Antofagasta), kept epidemiological silence and did not report suspected cases compatible with EI infection in their equine population. The index cases may not be the source of the virus, they act to amplify the virus and serve as a source of infection to other horses in the

cohort. The severity of the disease depends primarily on the immunological condition of the horses at the time of exposure, the environment, the stress created by continuing to work or being trained (Cullinane and Newton 2013). Equine influenza is a self-limiting disease, and the virus does not persist in horses that have recovered. It is thought that influenza virus persists in endemic populations by low-grade circulation with small occasional outbreaks (Glass *et al* 2002).

The 2018 EI H3N8 subtype outbreak in Chile was caused by reduced vaccination and poor herd immunity of Chilean equids, particularly Pure Breed Chilean horses. Regulations that will require strategic vaccination of horses that move permanently, or temporarily for sporting purposes are being evaluated.

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