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Multifocal outbreak of equine influenza in vaccinated horses in Argentina in 2018: Epidemiological aspects and molecular characterisation of the involved virus strains

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Summary

Background: Equine influenza is an important cause of respiratory disease of horses worldwide. The equine influenza virus (EIV) undergoes antigenic drift through the accumulation of amino acid substitutions in the viral proteins, which may lead to vaccine breakdown.
Objectives: To describe the epidemiological findings and the molecular characteristics of the EIV detected during the multifocal outbreak that occurred in Argentina between March and July 2018 and evidence a vaccine breakdown.
Study design: Observational, descriptive study.
Methods: Virus was detected in nasopharyngeal swabs using real-time reverse transcriptase PCR (RT-PCR). Nucleotide and deduced amino acid sequences of the haemagglutinin (HA) and neuraminidase (NA) genes were obtained from EIV positive nasopharyngeal swabs, and phylogenetic analysis was undertaken. Amino acid sequences were compared against the current World Organisation for Animal Health (OIE)-recommended Florida clade 1 vaccine strain and strain components of vaccines used in Argentina. Serum samples were tested using haemagglutination inhibition test.
Results: Equine influenza virus infection was confirmed using real-time RT-PCR and serological testing. The phylogenetic analysis of the HA and NA genes revealed that all the EIV identified during the outbreak belong to the H3N8 subtype, Florida clade 1. Multiple amino acid changes, some of them at antigenic sites, were observed in the circulating virus when compared with the strains included in the most commonly used vaccine in Argentina. Seventy-six percent of the affected horses had been vaccinated with this vaccine, suggesting the occurrence of vaccine breakdown.
Main limitations: The study does not include antigenic characterisation and full genome sequencing of Argentinian strains, that could provide additional information.
Conclusions: The occurrence of this multifocal equine influenza outbreak in regularly vaccinated horses is a field evidence of vaccine breakdown, reinforcing the necessity of keeping vaccine strains updated according to OIE recommendations. It also underlines the importance of the implementation of appropriate quarantine measures and restriction of horse movement in the face of disease.

Keywords: horse; H3N8; Florida clade 1; haemagglutinin; neuraminidase; South America

Introduction

Equine influenza (EI) is considered the most important cause of respiratory disease of horses in economic terms. The highly contagious nature of the causative agent, equine influenza virus (EIV), facilitates a rapid spread among susceptible horses, with their consequent withdrawal from equestrian activities [1,2]. The H3N8 subtype of EIV was first detected in 1963 in Florida, USA [3,4]. Phylogenetic analysis of the haemagglutinin (HA) gene showed that the H3N8 EIV evolved as a single lineage until the mid-1980s, when it diverged into two evolutionary different lineages, American and European [5–7]. Subsequently, the American lineage evolved into South American, Kentucky and Florida sublineages [8]. Nowadays, the Florida sublineage is predominant and has diverged into Florida clade 1 and Florida clade 2 [5]. Florida clade 1 viruses have been isolated in many countries [1,5,9–14] including Chile, Brazil, Uruguay and Argentina [13,15,16]. Florida clade 2 strains are commonly found in Europe but have been also identified in Asian countries [17–19].

Like other Influenza A viruses, EIV undergoes antigenic drift leading to vaccine breakdown [20,21]. Since 2010, the OIE-ESP recommendations have included both Florida clades 1 and 2 sublineages strains in EI vaccines [22,23]. In Argentina, EI vaccination is mandatory for mobile or congregate equine populations, and due to the characteristics of the commercial vaccines available in the country, booster vaccination is required every 3 months [24]. Most horses in Argentina are regularly vaccinated with an imported vaccine that contains the A/eq/Kentucky/1997 EIV strain, while only a small proportion of horses is vaccinated with other local vaccines which contain updated strains according to the OIE-ESP recommendations (A/eq/Argentina/E2345-1/2012 and A/eq/Meath/2007).

The aim of this work was to describe the epidemiological and molecular characteristics of the EIV detected in Argentina during the multifocal outbreak that occurred between March and July 2018 and analyse the causes that could have contributed to the unprecedented magnitude reached.

Materials and methods

Sample collection

Nasopharyngeal swabs (NS) in viral transport media (n = 74) and whole blood samples (n = 54) were collected from horses showing clinical signs of acute respiratory disease. Whole blood samples from 26 horses were obtained during the convalescent period of the disease and from 20 in-contact horses that had not showed clinical disease.

Equine influenza virus detection

Viral RNA was extracted from NS using the QIAamp Viral RNA mini kit[a], according to the manufacturer's instructions. A pan-reactive influenza type A real-time reverse transcriptase PCR (RT-PCR) targeting the matrix gene was performed for the detection of EIV, as previously described [13].

HA and NA sequencing and phylogenetic analysis

The complete HA (1700 nt) and NA (1410 nt) genes from 12 EIV real-time RT-PCR positive NS collected in different premises were amplified using the OneStep RT-PCR kit[a] and the primers have been described previously[9,25].

The PCR products were purified using the ExoStar kit[b], according to the manufacturer's recommendations and submitted for sequencing at the Unidad de Genómica, Instituto de Biotecnología, INTA.

The obtained HA and NA nucleotide sequences were edited with the BioEdit software v7.0.9.0 [26] and were aligned using ClustalW in the software package of BioEdit, along with HA and NA complete sequences representative of different H3N8 EIV lineages available at the Influenza Research database (IRD) and the global initiative on sharing avian influenza data (GISAID) database (Supplementary Item 1). Phylogenetic trees were inferred by the maximum likelihood method using PhyML v3.1 software [27]. The best fit evolutionary models of nucleotide substitution were estimated with the jModelTest v2.1.6 software [28].

Amino acid analysis

The deduced amino acid sequences of the HA and NA of the EIV identified during the described outbreak were aligned and analysed with representative strains' sequences obtained from the IRD and GISAID using BioEdit v7.0.9.0 software [26].

Equine Influenza antibody detection and quantification

To determine the EIV antibody level, serum samples were tested using a haemagglutination inhibition test (HI) against A/eq/Argentina/E-2345-1/2012 (Florida clade 1 strain), in accordance with the standard procedure [29]. Sera were pretreated with potassium periodate to remove nonspecific haemagglutinins and then inactivated at 56°C for 30 min. A seroconversion was defined as a ≥4-fold increase in HI antibody titre between the acute and convalescent serum sample. In addition, antibody levels ≥64 were considered 'high', between 16 and 32 'moderate' and ≤8 'low'.

Results

Outbreak description

Between 23 March and 10 July 2018, EI was clinically observed in horses stabled in premises located at geographically distant locations in Argentina (Fig 1). The disease occurred among thoroughbred and non-thoroughbred horses, including jumping and polo (Table 1). The virus was first detected in a racecourse in Mendoza province, on 23 March, where 70% of the horses were clinically affected. The official notification of this occurrence to the OIE was made by the National Animal Health Authorities (SENASA) on 4 April [30]. Between 31 March and 12 April, clinical cases were observed in San Isidro and Palermo racecourses, located in the Buenos Aires city area. According to the data reported by the facilities' veterinarians, the morbidity of disease was 70% in Palermo and 10% in San Isidro. From 11 April to 2 May, clinical cases were observed among polo horses, in three different premises, located in Capilla del Señor, Pilar and Hurlingham, all in Buenos Aires province. Between 27 April and 10 July, horses from five jumping clubs, located in the surroundings of Tandil, Pilar and San Miguel, in Buenos Aires province, were affected. In one of these jumping clubs, the morbidity reached 48%. Moreover, SENASA reported clinical cases consistent with EI in other four different racecourses, located in La Plata city (Buenos Aires province), Chubut and Santa Fe provinces, plus in horses from the army in San Juan province.

Clinically affected animals ranged from 1 to 6 years of age. Clinical signs were characterised by pyrexia, coughing and nasal discharge, initially serous and then mucopurulent. Sixty-one percent of the affected horses had received at least one dose of vaccine in the previous 3 months; of these 76% had been vaccinated with a vaccine containing the American strain A/eq/Kentucky/1997 and 24% with a vaccine containing the updated EIV strains (Florida clade 1, A/eq/Argentina/E2345-1/2012 and the Florida clade 2, A/eq/Meath/07). The remaining 39% of the affected horses were unvaccinated or had an out-of-date vaccination history.

The geographical distribution, type of affected premises and vaccination status of the horses are summarised in Figure 1 and Table 1.

★ Santiago, Chile, (1) Mendoza, (2) San Isidro and Palermo, (3) Capilla del Señor, Pilar, Hurlingham and San Miguel, (4) Tandil. Reported by SENASA (5) La Plata, (6) Chubut, (7) Santa Fe and (8) San Juan.

Fig 1: Geographical distribution of premises affected during equine influenza outbreak in Argentina in 2018.

Equine influenza virus detection

EIV was detected using real-time RT-PCR in 43 out of 74 (58%) NS collected from affected horses from Mendoza (n = 10), San Isidro (n = 7), Palermo (n = 5), Capilla del Señor (n = 1), Hurlingham (n = 3), Tandil (n = 4), Pilar (n = 2) and San Miguel (n = 11) (Table 1).

HA and NA phylogenetic analysis

The nucleotide sequence of the HA and NA genes of the 12 EIV identified in seven premises were obtained and the resulting sequences were introduced into the GISAID database [31] (Table 1). The maximum likelihood phylogenetic trees for HA and NA genes are shown in Figures 2 and 3, respectively.

Phylogenetic analysis of the HA gene indicated that all EIV strains detected in the 2018 EI outbreak in Argentina grouped into a monophyletic clade within the Florida clade 1 (Fig 2), together with 2018 isolates from Chile (A/eq/Concepcion/RO1C/2018) and Scotland (A/eq/EastLothian/2/2018); and are related to viruses identified in the USA in 2016 (A/eq/Georgia/121362-16/2016) and Japan in 2017 (A/eq/Yokohama/aq100/2017). The strains detected in the outbreak form a monophyletic group, slightly different from the one that included strains detected in Argentina in 2012. The phylogenetic tree shows that the strain A/eq/Kentucky/97, which is included in the most used vaccine in Argentina, groups in the American lineage quite far from the circulating Florida clade 1 strain.

TABLE 1: Summary of the EIV outbreak that occurred in Argentina in 2018

Premise number	Date	Location	Premises Type	Clinically affected (%)	Confirmed cases/ Symptomatic analysed	Up to date vaccination records	Isolate name	HA and NA GISAID acc N°
1	23/03/2018	Mendoza	Racecourse	70%	10/10	Not available	A/eq/Argentina/E151-1/2018	EPI_ISL_335219
2	31/03/2018	San Isidro	Racecourse	10%	7/11	Vaccinated	A/eq/Argentina/E161/2018	EPI_ISL_335220
							A/eq/Argentina/E167-1/2018	EPI_ISL_335221
							A/eq/Argentina/E167-2/2018	EPI_ISL_335222
3	31/03/2018	Palermo	Racecourse	70%	5/5	Vaccinated	A/eq/Argentina/E162/2018	EPI_ISL_335223
							A/eq/Argentina/E166-1/2018	EPI_ISL_335317
							A/eq/Argentina/E166-2/2018	EPI_ISL_335318
4	11/04/2018	Capilla del Señor	Polo club	NA	1/2	Vaccinated	NA	NA
5	13/04/2018	Hurlingham	Polo club	NA	3/3	Vaccinated	A/eq/Argentina/E200-1/2018	EPI_ISL_335319
6	27/04/2018	Tandil	Jumping club	NA	4/5	Vaccinated	A/eq/Argentina/E267-3/2018	EPI_ISL_335320
7	02/05/2018	Pilar	Polo club	NA	1/4	Vaccinated	NA	NA
8	21/06/2018	San Miguel	Jumping club	48%	5/6	Vaccinated	A/eq/Argentina/E434-5/2018	EPI_ISL_335321
							A/eq/Argentina/E434-6/2018	EPI_ISL_335338
9	26/06/18	San Miguel	Jumping club	8%	5/12	Vaccinated	NA	NA
10	06/07/18	Pilar	Jumping club	NA	1/1	Vaccinated	NA	NA
11	10/07/18	San Miguel	Jumping club	30%	1/6	Vaccinated	A/eq/Argentina/E474-12/2018	EPI_ISL_335339

Data presented include the geographical distribution, type of affected premises, morbidity, vaccination status of the affected horses and the GISAID accession numbers of the virus isolates.
GISAID, global initiative on sharing avian influenza data; NA, not available data; HA and NA GISAID acc N°, Haemagglutininin and Neuraminidase GISAID accession number.

As for HA gene, the phylogenetic tree shows that the complete NA sequences of EIV detected during the 2018 Argentinian outbreak form a monophyletic group within the Florida clade 1 (Fig 3), together with strains isolated in Chile and Scotland in 2018 (A/eq/Concepcion/RO1C/2018 and A/eq/EastLothian/2/2018, respectively), related to viruses detected in USA during 2016 and separated from the ones detected in Argentina in 2012.

HA derived amino acid alignment

The derived HA amino acid sequences of the EIV identified in this study were aligned with A/eq/Argentina/E-2345-1/2012, a representative local strain, which is a component of vaccines manufactured in Argentina and with A/eq/Ohio/1/2003, an OIE-ESP recommended Florida clade 1 vaccine strain (Fig 4). In addition, they were also compared with A/eq/Kentucky/97, the strain present in the vaccine most used in Argentina.

In comparison with the representative strain isolated in Argentina in 2012, all the 2018 Argentinian strains differ by six amino acids: S6N, S47P, N63D (antigenic site E), N188T (antigenic site B) and A-14T and T-13I, both of them located at the predicted peptide signal sequence. When compared with A/eq/Ohio/1/2003, in addition to those described above, strains isolated in 2018 possess six additional substitutions: G7D, R62K (antigenic site E), D104N, A138S and V223I and K-14T located at the predicted signal sequence. Compared with A/eq/Kentucky/97, seventeen amino acids substitutions were found. Besides the changes described above, the substitutions T30S, V58I, V78A (antigenic site E), N159S (antigenic site B), S272V and E323V were also observed. The amino acid changes A and S observed at the positions 78 and 159 respectively, are considered phenotype markers of the clade 1 viruses. Regarding the derived amino acid sequence of HA2 gene, four amino acid differences were obseved between the Argentinian 2012 and 2018 EIV isolates: K121R, Q125L, D145N and V198I. In comparison with the OIE-ESP prototype Florida clade 1 strain, all the substitutions described above were present but the change V198I.

Comparative analysis of the amino acid sequence of the strains circulating in Argentina in 2018 showed that these viruses have 100% sequence identity with a strain detected in Chile in January 2018 (A/eq/Concepcion/RO1C/2018) and in Scotland in February 2018 (A/eq/East Lothian/2/2018) and 99% sequence identity with strains isolated in Japan in 2017 and USA in 2016 (A/eq/Yokohama/aq100/2017 and A/eq/NewYork/1358572016, respectively).

NA derived amino acid alignment

The derived NA amino acid sequences of the 2018 Argentinian EIV were compared with the representative local strain isolated in 2012 (A/eq/Argentina/E-2345-1/2012) and the OIE-ESP recommended Florida clade 1 strain (A/eq/Ohio/1/2003). Twelve and 15 amino acid changes were found compared with A/eq/Argentina/E-2345-1/2012 and A/eq/Ohio/1/2003, respectively (Fig 5). From these, 10 changes were identical compared with the 2018 Argentinian strain with both reference strains: A13T, N21S, G47E, T68I, R76K, V147I, R252K, D258N, P397Q and T434K. Two additional substitutions were observed between 2018 and 2012 Argentinian strains: M8I and S205N. When compared with the OIE-ESP recommended Florida clade 1 vaccine strain, five further substitutions were found: V35A, R260K, E271G, S337N and G416E.

The Argentinian strains circulating in 2018 share 100% sequence identities with the EIV detected in Chile and Scotland in 2018 and 98% with strains isolated in USA in 2016.

Equine influenza antibody detection and quantification

The HI antibody levels found are summarised in Figure 6. Among the horses with clinical signs, 30% (16/54) of them had no or low antibody levels, 33% (18/54) had moderate and 37% (20/54) had high antibody levels at the acute stage of the disease. In 61% (33/54) of these animals, EIV was detected in NS using real-time RT-PCR, and among them, 43% (14/33) had no or low antibody levels, 33% (11/33) had moderate and 24% (8/33) had high antibody levels at the time of NS sampling. Convalescent serum samples were obtained from 26 of these 33 horses and all of them exhibited a significant increase in the HI antibody levels (data not shown). Regarding the horses in which the virus was not detected (21/54), antibody level was $\leq$8 in 10% (2/21), moderate in 33% (7/21) and high in 57% (12/21) of them (Fig 6). On the other hand, the analysis of serum samples from the 20 animals which did not show any clinical signs but were in contact with affected horses, showed that 5% (1/20) of them had low, 40% (8/20) moderate and 55% (11/20) high antibody titres. These 20 horses had been vaccinated, within the previous 3 months, with a vaccine which contained updated EIV strains (Florida clades 1 and 2 strains).

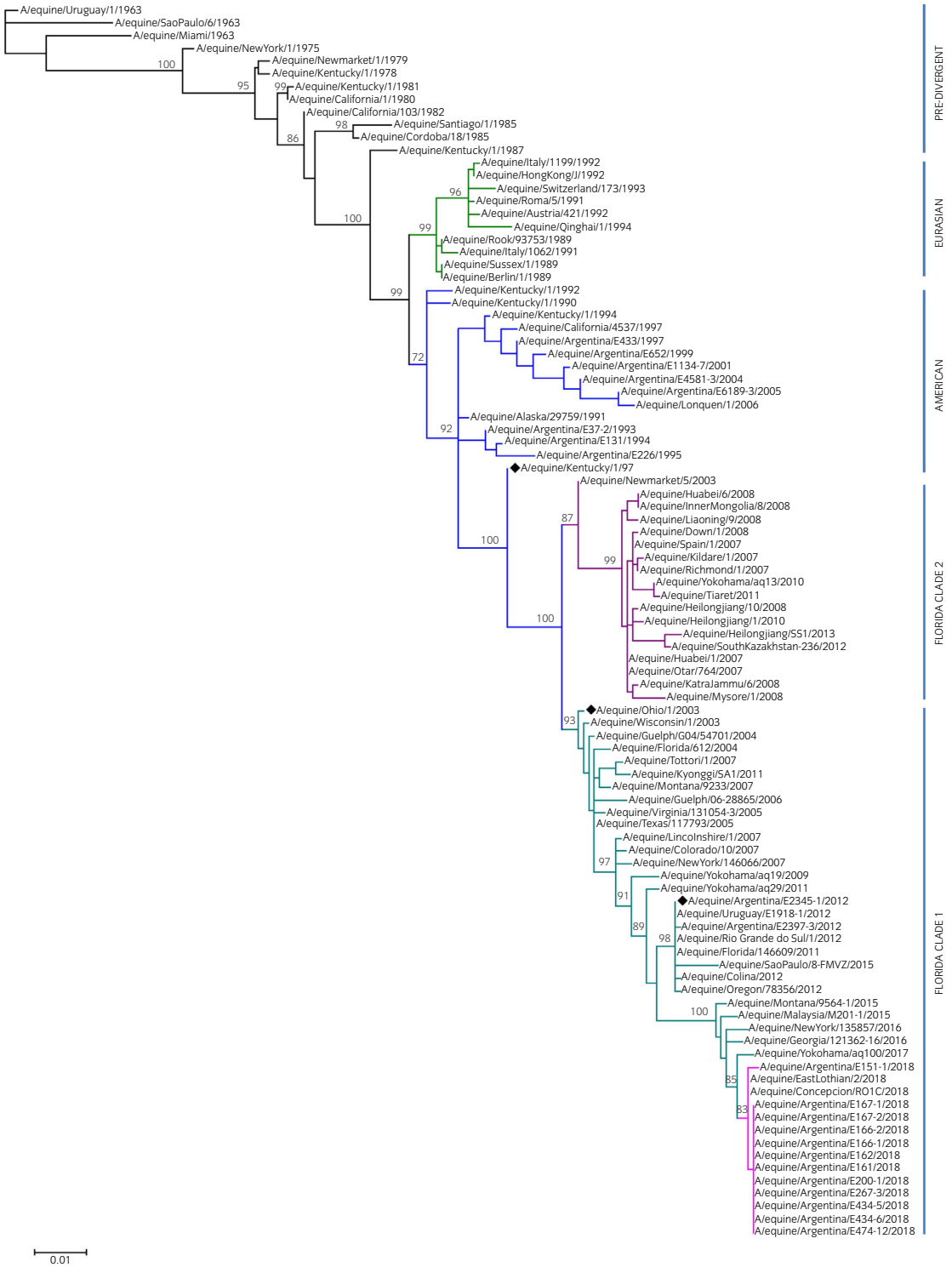

Fig 2: Maximum likelihood phylogenetic tree of the HA gene of H3N8 EIV. Bootstrap values obtained after 1000 replicates are shown at major nodes. Magenta branches correspond to 2018 Argentinian strains. ♦ represents the OIE-ESP Florida clade 1 recommended strain (A/eq/Ohio/1/2003) and the local strain included in vaccines (A/eq/Kentucky/1997 and A/eq/Argentina/E2345-1/2012).

Discussion

Equine influenza virus was the cause of a major outbreak of respiratory disease among Thoroughbred and non-thoroughbred horses in Argentina between March and July 2018. EIV infection was identified among horses in 16 facilities located in six different provinces around the country. Six of the affected facilities were Thoroughbred racecourses while three and seven were polo and horse jumping

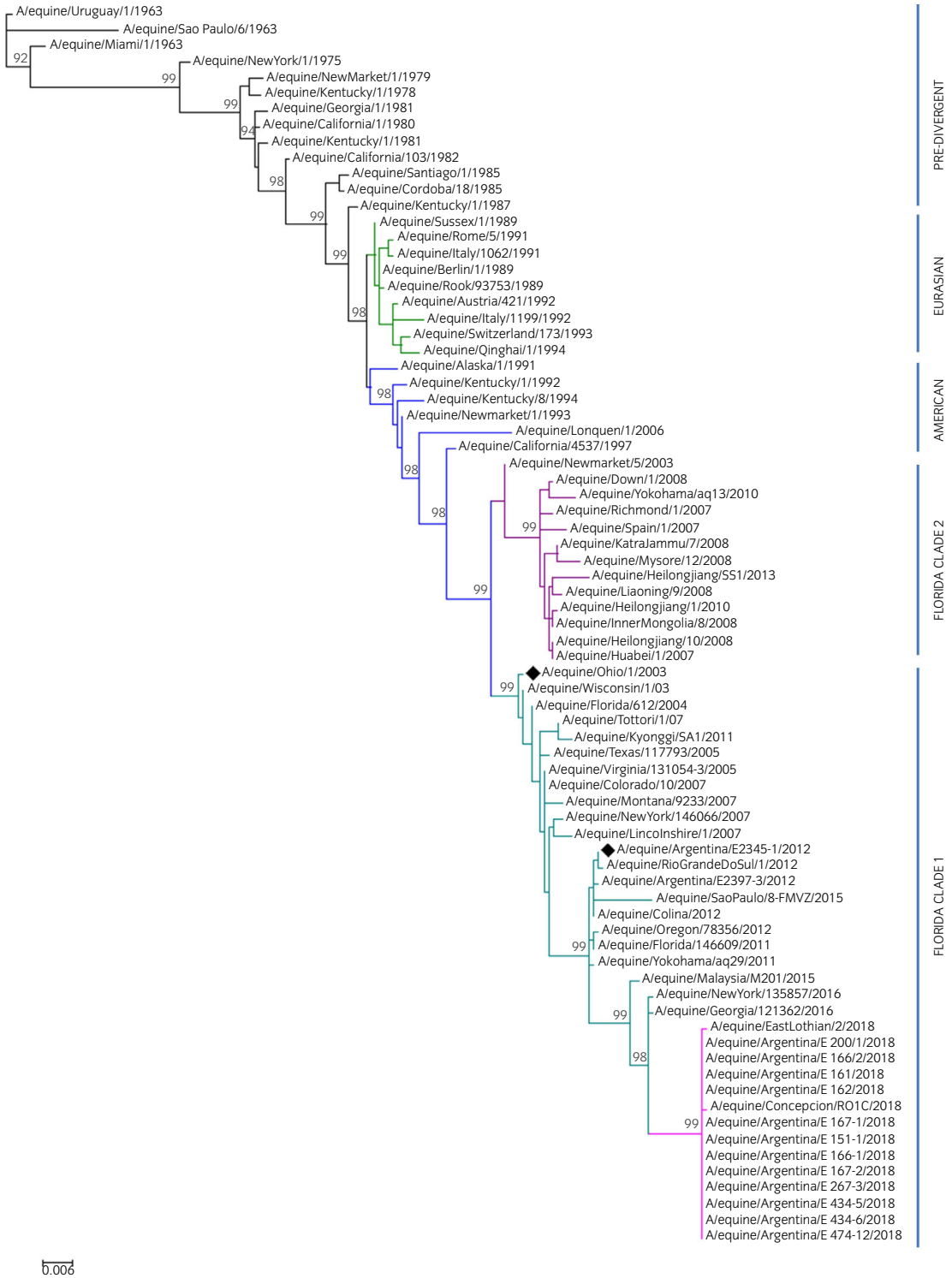

Fig 3: Maximum likelihood phylogenetic tree of the NA gene of H3N8 EIV. Bootstrap values obtained after 1000 replicates are shown at major nodes. Magenta branches correspond to 2018 Argentinian strains. ♦ represents the OIE-ESP Florida clade 1 recommended strain (A/eq/Ohio/1/2003) and the local strain included in vaccines (A/eq/Argentina/E2345-1/2012).

clubs, respectively. Large and multifocal outbreaks of EI are often associated with the commingling of horses at equestrian events and their subsequent dispersal over a wide geographical area [21,32]. During this outbreak, the movement of horses, competitions and equestrian events were not officially restricted, favouring the spread of the virus throughout the country.

Equine influenza virus was diagnosed using real-time RT-PCR and subsequently characterised using HA and NA genes nucleotide sequencing.

	Amino acid number																				
	HA1																	HA2			
Strain name	−14	−13	−6	7	30	47	58	62	63	78	104	138	159	188	223	272	323	121	125	145	198
A/eq/Kentucky/1/1997	K	T	S	G	T	S	V	R	N	V	D	A	N	N	V	A	E	N/A	N/A	N/A	N/A
A/eq/Ohio/1/2003	•	•	•	•	S	•	I	•	•	A	•	•	•	•	•	V	V	K	Q	D	I
A/eq/Argentina/E2345-1/2012	A	•	•	D	S	•	I	K	•	A	N	S	S	•	I	V	V	•	•	•	V
A/eq/NewYork7135857/2016	T	•	N	D	S	P	I	K	•	A	N	S	S	T	I	V	V	R	L	N	•
A/eq/Yokohama/aq100/2017	T	I	N	D	S	P	I	K	•	A	N	S	S	T	I	V	V	R	L	N	•
A/eq/East Lothian/2/2018	T	I	N	D	S	P	I	K	D	A	N	S	S	T	I	V	V	R	L	N	•
A/eq/concepcion/RO1C/2018	T	I	N	D	S	P	I	K	D	A	N	S	S	T	I	V	V	R	L	N	•
A/eq/Argentina/E151-1/2018	T	I	N	D	S	P	I	K	D	A	N	S	S	T	I	V	V	R	L	N	•
								Site E					Site B								

Fig 4: Alignment of the predicted HA amino acid sequences of the Argentinian 2018 strains against Florida clade 1 OIE-ESP reference strain (A/eq/Ohio /1/2003) and strains included in vaccines used in Argentina (A/eq/Kentucky/1997 and A/eq/Argentina/E2345-1/2012). Amino acid residues are numbered from the serine residue located downstream of the predicted signal sequence. Amino acid identity with A/eq/Kentucky/1997 in HA1 and A/eq/Ohio/1/2003 in HA2 is represented with a dot. N/A: A/eq/Kentucky/1997 HA2 nucleotide sequence information is not available at the IRD or global initiative on sharing avian influenza databases.

	Amino acid number																
Strain name	8	13	21	35	47	68	76	147	205	252	258	260	271	337	397	416	434
A/eq/Ohio/1/2003	I	A	N	V	G	T	R	V	N	R	D	R	E	S	P	G	T
A/eq/Argentina/E2345-1/2012	M	•	•	A	•	•	•	•	S	•	•	K	G	N	•	E	•
A/eq/NewYork7135857/2016	•	•	•	A	E	•	K	•	•	K	N	K	G	N	•	E	K
A/eq/East Lothian/2/2018	•	T	S	A	E	I	K	I	•	K	N	K	G	N	Q	E	K
A/eq/Concepcion/RO1C/2018	•	T	S	A	E	I	K	I	•	K	N	K	G	N	Q	E	K
A/eq/Argentina/E151-1/2018	•	T	S	A	E	I	K	I	•	K	N	K	G	N	Q	E	K

Fig 5: Alignment of the predicted NA amino acid sequences of the Argentinian 2018 strains against Florida clade 1 OIE-ESP reference strain (A/eq/Ohio/1/2003) and strains included in vaccines used in Argentina (A/eq/Argentina/E2345-1/2012). Amino acid identity is represented with a dot.

Phylogenetic analysis of the HA and NA genes showed that the viruses identified in 2018 in Argentina belong to the Florida clade 1 sublineage, and were related to viruses circulating in the USA in 2016, in Japan in 2017 and in Chile and Scotland in 2018; sharing more than 99% of similarity in the deduced amino acid sequences. The EIV strains that circulated in South America in 2012, also grouped within the Florida clade 1 sublineage, are however slightly different from the ones detected during the present outbreak, suggesting an independent reintroduction of the virus. A major contributing factor in the dissemination of EIV around the world is horse movement, despite mandatory vaccination programmes and quarantine procedures [33]. Early in 2018, EIV infection was detected in Chile and notified to the OIE, and the virus was characterised as belonging to Florida clade 1 sublineage, with 100% of amino acid sequence identity with the one causing the later EI outbreak in Argentina [30,34]. Considering that the movement of horses between Chile and Argentina is frequent for competition, touristic and leisure purposes, the source of virus for Argentinian horses could have been the introduction of subclinically infected horses from Chile. During the EI outbreak in Argentina, National Animal Health Authorities from Uruguay cancelled the movement of horses between both countries. However, on 11 June 2018, the occurrence of an EI outbreak in Uruguay was reported to the OIE [30,35].

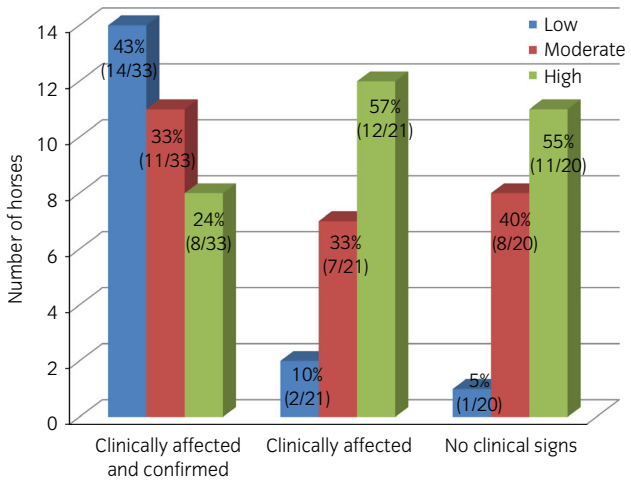

Fig 6: H3N8 EIV antibody level obtained for clinically affected and equine influenza positive (confirmed) horses, clinically affected horses and horses without clinical signs.

The significant increase in the H3N8 EIV antibody titres observed between the acute and convalescent serum samples was also an indirect evidence of the recent exposure to EIV in affected horses. Surprisingly, serological testing shows that 57% of the animals had moderate to high antibody titres (≥32) at the time of the detection of EIV in NS. A possible explanation of that fact could be the occurrence of vaccine breakdown, related to the use of vaccines that contain out-of-date EIV strains. The efficacy of vaccines relies on the antigenic relatedness between the vaccine and the circulating field strains [33,36,37]. As antigenic drift in the HA and NA genes could result in amino acid changes in the respective proteins, the antigenicity of the virus may suffer changes [2]. Equine Influenza vaccine breakdown has been described in Japan among racehorses vaccinated 3 months before the occurrence of an outbreak, and in Ireland in a multifocal outbreak of EI [21,38]. During the EI outbreak in Argentina, 61% of the affected horses had up-to-date vaccination records, in concordance with SENASA regulations [24]. Among them, 76% had been vaccinated with a vaccine containing the old A/eq/Kentucky/1997 strain, which is phylogenetically distant from the Argentinian 2018 EIV strains. The analysis of the HA amino acid sequences showed that between A/eq/Kentucky/97 and the currently circulating strains, there are 16 amino acid substitutions, five of them located at antigenic sites, two at antigenic site B and three at antigenic site E. These amino acid substitutions are critical,

especially those located at antigenic site B at the top of the HA1 molecule; any amino acid change could compromise viral antigenicity and consequently vaccine efficacy [39]. Moreover, there is scientific evidence that the American strain A/eq/Kentucky/97 groups in an antigenically distinct group from the Florida clade 1 strains currently circulating [40]. Although 39% of the infected horses during the outbreak were unvaccinated or had an out-of-date vaccination record, we do not have enough information to correlate vaccination status with disease severity.

It is important to point out that 24% of the infected horses had been vaccinated with an updated vaccine containing Florida clade 1 and 2 strains (A/eq/Argentina/E2345-1/2012 and A/eq/Meath/2007, respectively); according to veterinary reports, these horses presented only mild respiratory clinical signs. Moreover, 20 horses, vaccinated with the same vaccine and in contact with diseased animals, remained healthy, without any clinical manifestation of disease. This fact could suggest a relationship between the vaccine used and the severity of clinical signs. Comparison of the HA of strains circulating during the described outbreak and the Florida clade 1 strain contained in the updated vaccine used in Argentina (A/eq/Argentina/E2345-1/2012), shows that there are six amino acid substitutions, one of them at antigenic site B and another at antigenic site E. Considering that the strains used in this vaccine and those circulating in Argentina in 2018 belong to the Florida clade 1, the amino acid changes that occurred in the HA and NA between both strains would not affect vaccine efficacy. Nevertheless, the antigenic characterisation of the 2018 EIV strains, which is ongoing, could add more information to help interpret the epidemiological and clinical outcome of this EI outbreak, that occurred among horses of different housing and training conditions, age and immunological status.

The importance of the timely collection of NS for virus detection and isolation has been widely described. Delays in the time of sampling led to difficulties to establish an association between EI infection and antibody titres at the time of virus exposure [32]. This could explain the fact that 90% of the animals with clinical signs but no virus detection in NS, presented moderate to high antibody titres, showing a serological profile similar to that observed in horses with no clinical signs.

In conclusion, EIV H3N8 subtype of the Florida clade 1 sublineage was the cause of a severe outbreak of EI influenza that occurred in vaccinated and unvaccinated horses in Argentina in 2018. Given the substantial economic losses that EI produces in the equine industry and considering the importance of national and international transport of horses in the spread of EIV, it is crucial that horse vaccine producer companies incorporate updated strains of EIV in line with the OIE-ESP annual recommendations. In addition, SENASA should only promote the commercialisation of biological products that meet the appropriate requirements of efficacy. Fulfilling appropriate vaccination programmes, using vaccines that contain updated strains, accompanied with proper quarantine procedures and continued epidemiological surveillance, appears to be the key consideration in order to diminish the risk of new outbreaks of this disease.

Author's declaration of interests

No competing interests have been declared.

Ethical animal research

Samples were collected from horses presenting respiratory signs, as part of the health monitoring of them.

Owner informed consent

The owners gave oral informed consent for their horses' inclusion in the study.

Data availability statement

The sequence data have been submitted to the GISAID EpiFlu databases under the accession numbers EPI_ISL_335219 to EPI_ISL_335223, EPI_ISL_335317 to EPI_ISL_335321, EPI_ISL_335338 and EPI_ISL_335339.

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Authorship

C. Olguin-Perglione, M.A. Vissani and M. Barrandeguy conceived and designed the experiments. C. Olguin-Perglione, M.A. Vissani, F. Alamos and M.S. Tordoya performed all research experiments and data analysis. The manuscript was written by C. Olguin-Perglione and M.A. Vissani under the supervision of M. Barrandeguy.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1: Equine influenza viruses included in phylogenetic analysis.