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Short communication

Epidemiology and molecular detection of equine herpesviruses in western Algeria in 2011

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ABSTRACT

An episode of acute equine respiratory infection was reported in western Algeria (Tiaret province) between February and March 2011, affecting a large population of horses. Nasal swabs (n = 100) were taken from horses aged between 1 and 27 years, presenting with cough and mucopurulent nasal discharge. The prevalence of equine respiratory virus infections was examined using quantitative polymerase chain reaction (qPCR). One, or more, of four equine respiratory viruses were detected in the nasal swabs of 90 of 100 horses (90%) and the detection rate of equine herpesvirus type 1 (EHV-1), equine herpesvirus type 4 (EHV-4), equine herpesvirus type 2 (EHV-2) and equine herpesvirus type 5 (EHV-5) were 2%, 14%, 90% and 75%, respectively. Equine influenza virus and equine arteritis virus were not detected in any samples. Among the 90 infected horses, 70 were co-infected with EHV-2 and EHV-5 and 14 others were co-infected with EHV-4, EHV-2 and EHV-5. The present study shows a positivity rate of 97.3% for EHV-5 in young horses aged < 3 years; a finding which decreased with age. Viral load of EHV-5 was significantly higher in < 3 years whereas no effect of age was observed with EHV-2. The study shows that equine herpesviruses 1, 2, 4 and 5 are endemic in horse populations from Algeria as detected for the first time by qPCR.

1. Introduction

Respiratory diseases are responsible for important economic losses within the horse racing industry (Dynon et al., 2007). A wide range of causes, including bacterial and viral infections, as well as a number of non-infectious factors have been reported (Ko et al., 2013). Worldwide, the most commonly described viruses that cause respiratory syndrome are equine influenza virus (EIV), equine herpesvirus 1 (EHV-1), equine herpesvirus 2 (EHV-2), equine herpesvirus 4 (EHV-4), equine herpesvirus 5 (EHV-5), equine arteritis virus (EAV), equine rhinitis A (ERAV) and B (ERBV) (Dynon et al., 2007).

On the African continent, two subtypes of EIV (H7N7 and H3N8) were identified in horses in Algeria (Hans et al., 2012; Laabassi et al., 2015).

Whereas no evidence of EAV circulation in Algeria has been published, a recent study showed a seroprevalence of 7.5% in the horse population tested (Laabassi et al., 2014).

Equine herpesvirus 1 and EHV-4 are *Alphaherpesviruses* (Davison, 2010) that cause severe lytic infection of host cells and rapidly induce a cytopathic effect.

Equine herpesvirus 2 and EHV-5 belong to the genus *Percavirus* in the family *Gammaherpesvirinae* (Davison, 2010). Infections due to γ-EHV are associated with respiratory diseases, pharyngitis, enlarged lymph nodes, fever, lack of appetite and poor performance in this species (Fortier et al., 2010). An association between EHV-2 and EHV-5 during infection and clinical signs of nasal discharge, enlarged lymph nodes and coughing were reported (Dunowska et al., 2002). *Alphaherpesviruses* (EHV-1, EHV-4) may be detected along with γ-EHV in respiratory samples (Hue et al., 2014; Ko et al., 2013; Pusterla et al., 2013). No information are available concerning EHVs in Algeria.

The aim of this study is to investigate, for the first time in Algeria, a wide range of respiratory viruses through the use of molecular biology techniques on a substantial population of horses presenting with respiratory symptoms.

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2. Materials and methods

2.1. Sample collection

Nasal swabs (NS) were collected from horses with clinical signs of respiratory disease including mucopurulent nasal discharge and deep dry cough, between February and March 2011, from the National Stud of Chaou Chaoua, National Office of Equine and Camelin Livestock (ONDEEC), the Equestrian Club and the racecourse of Tiaret (West province of Algeria). The horse population studied (n = 100) was composed of 55 females and 45 males. This population was subdivided in four different groups aged between 1 and 27 years as follows: 37 young horses (< 3 years old), 20 young adult horses (4–9 years old), 21 adult horses (10–15 years old) and 22 old horses (over 15 years old). The majority of horses were Arabian thoroughbred and Barbe breeds, born and bred in Algeria. In this study, only five horses had been vaccinated against EIV, EHV-1 and EHV-4. After collection, swabs taken from the nasopharynx were placed in 5 mL of viral transport medium (VTM) consisting of phosphate-buffered saline (PBS), 5000 U/mL penicillin, 250 mg/mL amphotericin B and 2% v/v foetal bovine serum and transported immediately on ice to the laboratory, then stored at −20 °C until use.

2.2. Nucleic acid extraction

Nucleic acid were extracted from 140 µL of each sample with the QIAamp® RNA viral Mini Kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. Nucleic acids were eluted in a final volume of 50 µL and stored at −80 °C until used.

2.3. Polymerase chain reaction (PCR)

Quantitative PCRs for EHV-1, EHV-4, EHV-2 and EHV-5 were developed as previously described for EHV-2 based on the standard model AFNOR NF U47-600-2 (Doubli-Bounoua et al., 2016; Hue et al., 2014). Efficiency is for each qPCR comprised between 75% and 125%. Sensitivity and specificity of each method is greater than 95%. Each reaction was processed in a total volume of 25 µL containing 2 × Taqman® Universal PCR Master Mix (Life technologies, Saint-Aubin, France), primers and probes. Thermal cycling for EHV-1 and EHV-4 proceeded at 95 °C for 10 min, followed by 50 cycles of 94 °C for 15 s and 60 °C for 1 min (Diallo et al., 2007); for EHV-2 and EHV-5 proceeded at 94 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min (Hue et al., 2014).

Presence of EIV was tested by qRT-PCR for a portion of the matrix protein (M1) gene as previously described (Legrand et al., 2013). The qRT-PCR was performed in 25 µL reaction with 0.4 µM of each primer and 0.3 µM of probe using the OneStep RT-PCR kit (Qiagen) according to the manufacturer's instructions. The qRT-PCR program was 30 min at 50 °C, 15 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s at 56 °C and 45 s at 61 °C.

Quantitative PCR assays were performed on StepOnePlus™ Real-Time PCR systems (Life Technologies). Data were analysed using the StepOne™ software, version 2.2.2 (Life Technologies).

EAV RT-PCR targeted the ORF7 gene (N protein) as previously described (Balasuriya et al., 2002). The RT-PCR was performed in 25 µL volumes with 10 µM of each primer and 10 µM of probe using the QuantiTect™ Virus (Qiagen) according to the manufacturer's instructions. The RT-PCR program was 20 min at 50 °C, 5 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 45 s at 60 °C and was performed on Mastercycler ep realplex (Eppendorf, France).

2.4. Statistical analyses

Chi-square test was used to compare the percentage of viral detection between age groups and was performed with Winks SDA v.7.0.5 software (Texasoft, USA). Normality of continuous data distribution of viral load was evaluated using the Kolmogorov-Smirnov test. The different parameters being not normally distributed, viral loads were log-transformed before comparison among age groups by ANOVA and Tukey-Kramer post-hoc test (General Linear Model), with breed and gender as covariates (NCSS 9; NCSS, LLC, Kaysville, UT, USA; www.ncss.com). Values of $p < 0.05$ were considered statistically significant.

Table 1
Presence of equine respiratory viruses detected by PCR from 100 nasal swab samples collected from 100 horses with clinical respiratory symptoms.

Classified infection	Virus	No. detected
		Infection rate (%)
Single-detection[a]	EAV	0
	EHV-1	2
	EHV-2	90
	EHV-4	14
	EHV-5	75
	EIV	0
Unique-detection	EHV-2 only	19
	EHV-5 only	5
Double-detection	EHV-1, EHV-2	1
	EHV-2, EHV-5	70
Triple-detection	EHV-1, EHV-4, EHV-5	1
	EHV-2, EHV-4, EHV-5	14

EAV: equine arteritis virus; EHV: equine herpesvirus; EIV: equine influenza virus.
[a] Number of single positives includes each number of double and triple detection.

3. Results

3.1. Virus detection by PCR

By investigating the presence of EIV, EAV and EHV 1, 4, 2 and 5, four different equine respiratory viruses were detected; the overall detection rate was 90% (Table 1). The most prevalent pathogens were EHV-2 and EHV-5, which were detected in 90% and 75% of the horses, respectively. The presence of EHV-1 and EHV-4 were detected in 2%, and 14% of the cases, respectively. All the samples were tested negative for EIV and EAV. Furthermore, the percentage of EHV-4 was significantly more frequent ($p < 0.05$) in young horses (< 3 years old) than in young adult horses (4 to 9 years old) and older horses (over 15 years old) (Fig. 1A). The presence of EHV-2 and EHV-5 varied according to age as shown in Table 2. The detection of EHV-5 was significantly ($p < 0.05$) more frequent in yearlings (< 3 years old) than in the other three groups (Fig. 1A). Unique detection of EHV-5 was observed in 5% of the samples studied (Table 1). However, unique detection of EHV-2 was observed in 19% of the samples (Table 1). The percentage of EHV-2 was not significantly different among the different age groups (Fig. 1A)

3.2. EHV Co-detection by qPCR in nasal samples

Respiratory EHVs (EHV-1, EHV-2, EHV-4 and EHV-5) were detected in 90 horses (90%), and among those 90 horses, 70 (70%) presented co-infection as follows: 70 horses were co-infected with EHV-2 and EHV-5, and 1 horse with EHV-1 and EHV-2; while 14 horses were co-infected with EHV-2, EHV-4 and EHV-5; and 1 horse with EHV-1, EHV-2 and EHV-5 (Table 1).

In addition, the percentage of co-infections with EHV-2 and EHV-5 was significantly ($p < 0.05$) more frequent in yearlings (< 3 years old) than in young adult (4 to 9 years old) and older horses (over 15 years old) (Fig. 1B).

Based on the age of the horse, the same incidence 97.3% (36/37) for EHV-2 and EHV-5 was observed for the samples from yearlings (< 3 years old, Table 2), with unique detection of EHV-2 and EHV-5 in