Equine herpesvirus-1 and 4: investigating the disease humoral immunity profile among some Native, Arabian and Foreign horses

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ABSTRACT

A preliminary field-based investigative study was conducted for screening, possible detection and determination of equine anti-herpesvirus-1 and 4 antibodies in blood sera collected from 230 adult Native, Arabian and Foreign Qatari and Egyptian horses. Using commercial kits, 99% (120/121) of the screened serum samples belonging to the Qatari horses seroconverted to either EHV-1 or EHV-4 subtypes. All of the total antibody positive sera were also anti-EHV-4 antibody positive. 16% (20/120) of the total positive were anti-EHV-1 antibody positive and they were also anti-EHV-4 antibody positive. Only 0.06% (8 sera) of the positive serum samples were from horses of a history of vaccination against the two viruses. None of the 49 corresponding peripheral mononuclear cells, randomly selected from 3 ELISA positive groups were real time PCR positive for either EHV-1 or 4 specific primer sets. Screening results against total anti-EHV-1, 4 antibodies of the 109 Egyptian horses’ sera indicated positive anti-EHV 1, 4 antibodies in 21% (23 sera). While 65% (15 sera) of the positive samples were originating from vaccinated horses, 34% (8 sera) were not. Based on the epidemiology of EHV-1 and 4, their infection and pathogenic potentialities, the antibody threshold of the positive sera and the natural habitat of the candidate horses; the obtained results infer previous natural exposure to EHV and possible circulation of EHV-1, 4 among these animals.

Key Words: Equine herpesvirus, Equine herpesvirus-1 and 4, Arabian and Thoroughbred horses, Equine herpes virus antibody, Enzyme-linked antibody assay.

Abbreviations: Equine herpes virus (EHV), Equine herpes virus-1 (EHV-1), Equine herpesvirus-4 (EH-4), Arabian horses, anti-equine herpes virus antibodies (anti-EHV-Ab), Enzyme-linked antibody assay (ELISA).

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INTRODUCTION

Equine herpes virus (EHV) is a several highly contagious, clinical disease afflicts equids probably caused by either of the two closely related herpes viruses, equine herpes virus-1 (EHV-1) or equine herpes virus-4 (EHV-4) (AAEP, 2013). The etiology of EHV infection are members of the Alphaherpesvirinae subfamily of herpes viruses of the domestic horse, currently, and together with several other important veterinary viruses are related to the genus Varicellovirus, (Davison et al. 2009; ICTV, 2012). Among the nine worldwide known EHV, EHV-1, EHV-3 and EHV-4 were found to pose health risk on domesticated horses and significant economical impact on horse industry (AQHJ, 2011). Similarly, equine herpes virus type 2 (EHV-2) and equine herpes virus type 5 (EHV-5), members of the subfamily gammaherpesvirinae, are also widely spreading in equine populations (AAEP, 2013) and both of which can cause
respiratory tract infections of varying severity characterized by fever, lethargy, anorexia, nasal discharge and cough (Crabb et al., 1995).

EHV-1 and EHV-4 are of two antigenically distinct viral groupings (Kleiboeker et al., 2002). Although, polyclonal antibodies (PAb) to both viruses are highly cross-reactive to each other, serological discrimination between each Ab is possible, a step that is highly essential for quarantine and preventive measures (MVM, 2010).

It was found that by 2 years of age most horses seroconvert to EHV-4. Contrary to this, seroprevalence is less for EHV-1 (Allen, 2002). However, while first infection of the horse by EHV-1 is possible during weeks or months of age, it was observed that latency and disease reactivation are critical features of the disease epidemiology of EHV-1. Moreover, another strikingly feature, recent studies indicated that EHV-1 could jump species barrier to infect ruminants (Pagamjav et al., 2007).

Attempting to cast light on EHV situation in some equine species, a study was carried out to screen horses' blood sera for EHV-1 and EHV-4 antibodies targeting some animals located in Qatar and Egypt. Available data of breed type, age, vaccination and movement history were included.

MATERIALS AND METHODS

Animals:

 Apparently healthy stabled horses (121 in Qatar and 109 in Egypt premises) making a total of 230 were used for the investigation.

Samples collection from Qatari and Egyptian horses:

Volumes of 5 to 8 mL blood serum samples each were collected from the 109 Egyptian horses and 121 Qatari horses, where as simultaneously, equivalently, 121 whole blood-EDTA were collected from the same 121 Qatari horses using external jugular venipuncture method. Breed, age, previous vaccination and last trip history were also recorded.

Preparation of blood serum samples from Qatari and Egyptian horses:

A total of 230 serum samples were prepared according to the standard procedures after blood clotting at ambient environment and overnight incubation at 18 - 26°C controlled room temperature. All sera were collected individually into 2 mL cryovial and stored at -80°C facility till used.

Preparation of peripheral blood mononuclear cells from Qatari horses blood samples:

Peripheral blood mononuclear cells (PBMC) were prepared according to the standard procedures with minor modifications. Briefly, all blood-EDTA samples were centrifuged at 2000 rpm for 5 min at 20°C using Allerga X-22R centrifuge. About 1 mL of the tube contents RBC-contaminated buffy coat on top of the RBC layer was collected into sterile 15 mL centrifuge tube and diluted 1:6 using 5 mL sterile phosphate-buffered saline (PBS), pH 7.4. Then, 3 mL (1:2) histopaque solution (6.41g/dl polysucrose and 10.02g/dl sodium diatrizoate, density gradient gradient 1083±0.001 g/mL, Sigma) was carefully dispensed underneath the diluted buffy coat and the tube content was centrifuged at 2500 rpm for 10 min at 20°C. Then after, about 1 mL of the clear buffy coat on top of he histopaque phase was collected, transferred to a sterile centrifuge tube, and washed three times using 8X volume sterile PBS. Post the final washing, all PBMC pellets were re-
suspended into 1 mL PBMC preservation medium (70% RPMI 1640 medium, Sigms; 20% FCS, 10% dimethylsulphoxide) and stored gradually at -80°C till used.

**Determination and discrimination of anti-EHV-1, 4 antibodies in Qatari horses’ blood sera:**

An indirect solid-phase Enzyme-linked immunosorbent assay (ELISA) was used for quantitative detection and discrimination of the anti-EHV 1 and EHV-4 antibodies in the collected sera following the manufacturer (Svanova, Lot: 10-3100-02; P:00747, Sweden) procedure. Obtained results were validated, interpreted and recorded using BioTek Gen5 software-aided microtitre spectrophotometric device and a validated EHV-1, 4 indirect ELISA Ab Spreadsheet calculators.

**Determination of anti-EHV-1, 4 antibodies in Egyptian horse blood sera:**

The indirect solid-phase ELISA was used for quantitative detection of the anti-EHV 1-4 Ab in the collected sera following the manufacturer (Ingezim Rhino 1,4 HVE-K.1, Lot: 10-3100-02; P:00747, Spain) instructions. Obtained results were validated, interpreted and recorded using BioTek Gen5 software-aided spectrophotometric microtitre machine.

**Purification of EHV-1, 4 viral DNA from peripheral blood mononuclear cells Qatari horses’ samples:**

Fifty-six randomly selected PBMC samples previously collected from the Qatari horses were used for possible detection of EHV-1, 4 using High Pure Viral Nucleic Acid Kit, Lot 11858 874 001, Version 18 (Roche Diagnostics GmbH, Germany). All the purified EHV nucleic acid samples were stored shortly at -20°C till used.

**Real time polymerase chain reaction:**

The qualitative real time polymerase reaction (RT-PCR) was used for possible detection of EHV-1, 4 in the isolated viral DNA template according to Primer design™ genesig® Kit for equid herpesvirus-1 (EHV-1) and 4 (EHV-4) (Genesig Advanced kit handbook HB10.03.07, UK). Using Percision Plus TM 2x qPCR Master Mix, each 5 µL each DNA extract template sample, positive and negative controls, respectively, were added. Template amplification and PCR analysis was conducted through ABI 7500 Real Time Cycler was used for amplification and analysis of cDNA fragments using a program of 37°C for 10 min, UNG treatment; 95°C for 2 min, enzyme activation; 50 cycles-amplification of 95°C for 10 sec; denaturation and 60°C for 60 sec; data collection.

**RESULTS**

**Horses epidemiology:**

All of the candidate horses were sound to abnormal disease sign or to apparent EHV-1 and 4 clinical manifestations.

**Detection and discrimination of anti-EHV-1, 4 antibodies in Qatari horse’ blood sera:**

The profile of anti-EHV-1, 4 Ab is shown in Table 1. The total positive EHV-1, 4 sera were recorded. The discriminative Ab profile of each and the suspected cases were also demonstrated. None of the EHV-1, 4 Ab positive animals was found positive to EHV-1, 4 probing their respective PBMC cDNA template.

**Detection of total anti-EHV-1, 4 antibodies in Egyptian horses’ blood sera:**
The profile of total anti-EHV-1, 4 Ab is shown in Table 1. None of the positive sera was subjected to EHV-1 or EHV-4 discrimination Ab testing.

**Table 1:** Anti-EHV-1 and 4-antibody profile in the Qatari and Egyptian blood sera used throughout the investigation course

<table>
<thead>
<tr>
<th>Trait</th>
<th>Qatari horses (121)</th>
<th>Egyptian horses (109)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total positive</td>
<td>Percentage</td>
</tr>
<tr>
<td>EHV-1, 4 positive</td>
<td>120</td>
<td>99%</td>
</tr>
<tr>
<td>EHV-1 positive</td>
<td>20</td>
<td>16%</td>
</tr>
<tr>
<td>EHV-4 positive</td>
<td>120</td>
<td>100%</td>
</tr>
<tr>
<td>EHV-1 suspected</td>
<td>15</td>
<td>12%</td>
</tr>
<tr>
<td>EHV-4 suspected</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>EHV-cDNA positive</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

- ND: Not done

**Table 2:** Total anti-EHV antibody positive sera versus total EHV vaccination history of the Qatari and Egyptian horses

<table>
<thead>
<tr>
<th>Horses origin</th>
<th>Total EHV-1, 4 horses</th>
<th>Total EHV-1, 4 vaccinated</th>
<th>Percentage of vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qatar</td>
<td>120</td>
<td>8</td>
<td>0.06%</td>
</tr>
<tr>
<td>Egypt</td>
<td>23</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In the context of conducting a preliminary field-based investigation to screen, possible detection and determination of equine anti-herpesvirus-1 and 4 antibody picture in sera collected from 230 Arabian and Thoroughbred horses, it is obvious that some of the examined sera were positive for either EHV-1, EHV-4 or collectively to both.

The fact that 21% (23 sera) of the Egyptian horses, with unknown history of vaccination against EHV-1, 4 were seropositive for anti-EHV-1, 4 might exhibit prior exposure to these viruses.

On the other hand, with a vaccination history of 0.06% (8 horses) of the total positive samples, the 99% seroconversion rate of 120 of the screened serum samples belonging to the Qatari horses implies previous exposure to both serotypes. A clue of circulation of either virus subtypes among these horses could be postulated. The investigation finding that anti-EHV-4 antibodies were predominant representing 99% of the positive cases agreed with the previous studies of (Harless and Pusterla 2006), demonstrating prevalence of EHV-4 antibodies over that of EHV-1. Although the study observed suspected anti-EHV-1 antibodies among some of the Qatari horses’ sera (Table 4-2), the seroconverstion percentage of EHV-4 among these horses remains higher. Allen *et al.* (2004) had already attributed this to the superiority of the reactivation potentiality of the former virus type compared to that of EHV-1. The primary association of EHV-4 with upper respiratory disease especially in young horses (Patel and Heldens, 2005; Lunn *et al.*, 2009) might also explain the widely disseminating antibodies among horse.
Moreover, though the investigation did not include humoral antibody discriminative studies among the positive Egyptian horses, the molecular detection of EHV-4 viral genomes in the aborted fetal tissues further reinforces the previous raised conclusion.

Yet, molecularly no virus was detected in the PBMC of the anti-EHV-1 and 4 positive antibodies of the Qatari horses, a fact that could be due to latency (Crabb et al., 1995); the findings agreed with the epidemiological fact that exposure to EHV-4 is very common in young foals at early postnatal live (Patel and Heldens, 2005; Lunn et al., 2009).

The fact that equines play an important role in human live that they could play roles ranging from drafting to industrial agent signifies the attention that should be given to these animals. Horses significantly cross the boarders rendering them susceptible to EHV-1 and 4 infections. Considering the epidemiology of the disease among susceptible horses that they could develop latent infection (Allen et al., 2004), more attention should be bayed to apply sanitary measures dealing with EHV.

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REFERENCES


