Journal of Equine Veterinary Science 37 (2016) 11–16

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Short Communication

Surveillance of Encephalitis-Causing Arboviruses in Horses in South Korea

Hye-Young Jeoung [a], Sun-Ju Yang [b], Yun-ki Choi [b], Ji-Hye Lee [a], Hyun-Ji Seo [a], Sung-Hee Kim [a], Yun Sang Cho [a], Yong-Joo Kim [a], In-Soo Cho [a], Jee-Yong Park [a,*]

[a] Foreign Animal Disease Division, Animal and Plant Quarantine Agency, Anyang, Gyeonggi-do, Republic of Korea
[b] Equine Hospital, Korea Racing Authority, Gwacheon, Republic of Korea

ARTICLE INFO

Article history:
Received 23 July 2015
Received in revised form 14 October 2015
Accepted 2 November 2015
Available online 11 November 2015

Keywords:
Surveillance
Arbovirus
Zoonosis
Encephalitis
Horse

ABSTRACT

Surveillance was conducted in South Korea to look for evidence of infection with Eastern equine encephalitis virus (EEEV), Western equine encephalitis virus (WEEV), Venezuelan equine encephalitis virus (VEEV), West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and Japanese encephalitis virus (JEV) in horses from 2012 to 2013. The surveillance consisted of passive surveillance of testing horses with neurologic symptoms such as paralysis, incoordination, ataxia and circling, and active surveillance of testing for serologic evidence of infection in healthy horses. Passive surveillance was conducted for EEEV, WEEV, VEEV, WNV, SLEV, and JEV, and whole blood and/or brain samples received from 49 horses with neurologic signs were tested by polymerase chain reaction (PCR). Active surveillance was conducted for WNV and JEV, and 2,695 serum samples collected from horses across the country were tested for antibodies by IgM enzyme-linked immunosorbent assay (ELISA) and virus neutralization test (VNT), respectively. All samples tested by PCR were negative for EEEV, WEEV, VEEV, WNV, and SLEV, except for one whole blood sample (1/45) that was positive for JEV. All samples tested for WNV antibodies were shown to be negative. For JEV, because South Korea is endemic and horses in South Korea are vaccinated against JEV, various titers of antibodies for JEV were detected by VNT in 57.8% (737/1,274) and 58.9% (837/1,421) of the sera in 2012 and 2013, respectively. The surveillance provides evidence that supports the view that South Korea is free from EEEV, WEEV, VEEV, WNV, and SLEV. In addition, the surveillance scheme was shown to be able to identify JEV infections in the equine population and provide serologic data for JEV that could be used to improve the current vaccination program for JEV in horses.

1. Introduction

Equine encephalitis is characterized by signs of central nervous system dysfunction with moderate to high mortality [1], and arboviruses belonging to *Togaviridae* (genus *Alphavirus*) and *Flaviviridae* (genus *Flavivirus*) are the most common cause of equine viral encephalitis [2]. A number of alphaviruses including Eastern equine encephalitis virus (EEEV), Western equine encephalitis virus (WEEV), and Venezuelan equine encephalitis virus (VEEV) are distributed across the United States, whereas flaviviruses such as West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and Japanese encephalitis virus (JEV) are reported in many parts of the world except for SLEV, which has only been reported in the United States [2–4]. South Korea is considered to be free from infection with EEEV, WEEV, VEEV, WNV, and SLEV, although antibodies against WNV have been detected in migratory waterfowl [5]. Japanese encephalitis virus is endemic in South Korea and has been

* Corresponding author at: Jee-Yong Park, Animal and Plant Quarantine Agency, 175 Anyang-ro, Manan-gu, Anyang-si, Gyeonggi-do 430-824, Republic of Korea.
E-mail address: parkjyyunesy@korea.kr (J.-Y. Park).

0737-0806/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2015.11.004

detected in domestic animals such as horses, wild birds, mosquitoes as well as humans [6–9]. Many of these viruses such as EEEV, WEEV, VEEV, WNV, SLEV, and JEV are zoonotic and have the potential to emerge or reemerge in many parts of the world due to increased trade, increased movement of people and animals, and climate change [10–15]. Therefore, surveillance of arbovirus-associated encephalomyelitis was conducted in horses, which consisted of passive surveillance for EEEV, WEEV, VEEV, WNV, SLEV, and JEV by testing blood and/or brain tissue samples from horses with neurologic signs by antigen tests and active surveillance for WNV and JEV by testing sera with antibody tests. The surveillance was conducted to look for evidence of infection with arboviruses and to establish a test strategy that can quickly detect new introductions and elucidate the situation of equine encephalitis in South Korea.

2. Materials and Methods

2.1. Sample Collection

Passive surveillance was conducted for EEEV, WEEV, VEEV, WNV, JEV, and SLEV in clinically suspect cases of horses with neurologic signs, such as paralysis, incoordination, ataxia, and circling. Brain and/or whole blood samples from suspect cases were collected and submitted by field veterinarians to the Animal and plant Quarantine Agency for antigen testing. Samples were received between May and October of 2012 and 2013, which were from a total of 49 horses from Gyeongnam (12 affected horses from 11 farms), Busan (17 affected horses from 10 farms), Gyeongbuk (8 affected horses from 8 farms), Gyeonggi (9 affected horses from 7 farms), Jeonnam (2 affected horses from 2 farms), and Ulsan (1 affected horse from 1 farm). Brain samples consisting of forebrain, midbrain, hindbrain, cerebellum, brainstem, and spinal cord were collected from five horses during necropsy, and whole blood samples were received from 45 horses (Table 1) in plastic tubes with spray-coated K_2EDTA (BD, cat, 367488). Horses with samples positive by reverse transcription–polymerase chain reaction (RT-PCR) were considered to be positive cases. Active surveillance was conducted for WNV and JEV to look for evidence of infection in sera collected from clinically normal horses by antibody detection tests. Serum samples were collected through farm visits by the staff of the Korean Racing Authority, a public corporation under the Ministry of Agriculture, Food and Rural Affairs. A total of 2,695 serum samples were collected throughout South Korea (Fig. 1) between May and October of 2012 and 2013 (Table 2).

2.2. RNA Extraction and Identification of the Agents

Total viral RNA was extracted from samples using Maxwell 16 research instrument system (Promega, Medison, WI) with Maxwell 16 LEV simply RNA Tissue Kit (Promega, cat, AS1280) and Maxwell 16 LEV simply RNA Blood Kit (Promega, cat, AS1310), according to the manufacturer's instructions. Extracted RNA was tested using RT-PCR for EEEV, WEEV [16], and VEEV [17], and by multiplex real-time RT-PCR for WNV, JEV, and SLEV [18]. For positive controls, Korean JEV Anyang 300 strain, and WNV NY 385-99 strain (ATCC VR-1507), which was obtained from the American Type Culture Collection (ATCC, Manassas, VA), were used. For the rest of the viruses, the respective target genes were synthesized (Bioneer, South Korea) and used as controls.

2.3. Antibody Test for WNV

The serum samples were tested for WNV antibodies using ID Screen West Nile IgM antibody capture ELISA (ID VET, France). Samples were considered positive if the S/P % was $\geq$45%, according to the manufacturer's instructions.

2.4. Antibody Test for JEV

Serum samples were inactivated at 56°C for 30 minutes before analysis. Neutralizing JEV antibodies were detected in collected sera using an in vitro neutralization assay as described previously [19,20]. For in vitro neutralization assay, JEV (Anyang 300 strain) was maintained in Vero cells. All cell lines were grown in Dulbecco's modified Eagle's medium (GibcoBRL, Gaithersburg, MD) supplemented with 5% heat-inactivated fetal bovine serum (GibcoBRL) in a humidified 5% CO_2 atmosphere at 37°C.

For this study, different criteria were used for the determination of JEV infection for vaccinated and non-vaccinated horses. For horses with record of JEV vaccination, JEV infection was considered if VN titers were equal or above 1:640. This was based on previous reports that showed horses inoculated with inactivated JEV vaccines demonstrated antibody titers of less than 1: 640, which then increased to above 1:640 when the horses were experimentally inoculated with JEV 2 to 3 weeks after vaccination [19]. Japanese encephalitis virus–vaccinated horses with VN titers between 1:10 and 1:640 were considered to be due to vaccination and negative for antibodies to JEV if sera exhibited titers below 1:10. For horses that did not have record of vaccination, JEV infection was considered when titers were equal or above 1:160, based on previous report of horses with JEV infection demonstrating VN antibody titer from 1:160 to 1:640 [20]. Non-JEV–vaccinated horses with VN titers between 1:10 and 1:80 were considered suspect, and those with below 1:10 were considered negative.

3. Results

3.1. Passive Surveillance

Samples collected from horses with clinical signs indicative of arbovirus-associated encephalitis such as fever, depression, loss of appetite, paralysis, ataxia, circling, incoordination, and vision disorder [1,3,21] were tested. All samples collected from the 49 horses tested negative for EEEV, WEEV, VEEV, WNV, and SLEV by PCR. Japanese encephalitis virus was detected in one equine blood sample (1/45, sample number 13-2) by multiplex real-time RT-PCR. The positive sample was from a 5 -year-old mare located in Gyeongnam that had neurologic symptoms including hind paralysis. Japanese encephalitis virus was confirmed by sequencing PCR products by which the correct targets were amplified [22].