

Original Research Paper

Serological and Molecular Investigation of Middle East Respiratory Syndrome Coronavirus and Equine Herpesviruses in Horses at Southwestern Saudi Arabia

Ali S. Alqahtani

Department of Medical Laboratory Sciences, Faculty of Applied, Medical Sciences, Najran University, Najran, Saudi Arabia

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Email: almezherali@gmail.com

Abstract: Host reservoirs other than dromedaries for the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) still to be unknown. Equine Herpesviruses (EHVs) are widespread in equine populations worldwide. In this study, the presence of MERS-CoV, Equine Herpesviruses type 1 (EHV-1) and 4 (EHV-4) in horses in Najran, southwestern of Saudi Arabia was investigated. One hundred and thirty four blood samples collected from horses of different ages and sex. The samples tested for the presence of specific antibodies by ELISA and viral RNA and DNA by PCR. The ELISA results were positive for equine herpesviruses 1 and 4 with percent of 23.1 and 70.8% respectively; while all tested samples were negative for MERS-CoV. The PCR results showed that all samples were negative for all three viruses. As the history of the horses showed no previous vaccination or infection with equine herpesviruses, these results might refer to equine herpes subclinical infection. The results indicate also that horses were free from MERS-CoV infection.

Keywords: Equine, Herpesvirus, MERS, ELISA, PCR, Saudi Arabia

Introduction

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) first appeared in 2012 in Saudi Arabia and is now a worldwide concern (Zaki *et al.*, 2012). The World Health Organization (2019) had confirmed up to 2428 cases from 27 countries. The dyed cases were 791, most of them reported in Saudi Arabia. Infection through contact with dromedary camels is the major route in primary infection of human. However, human-to-human contact is the main rout of human infection (Sabir *et al.*, 2016; Reusken *et al.*, 2016). Previous serologic surveys of equines and ruminants did not indicate circulation of MERS-CoV among these animals (Adney *et al.*, 2016; Hemida *et al.*, 2013; Meyer *et al.*, 2015). The ability of the virus to jump species barriers and infect other animals is possible. The virus was capable of infecting cell cultures from mammals of many species (Chan *et al.*, 2013). Horses might be the highly susceptible host to MERS-CoV because of their great similarity in DPP-4 amino acids at positions critical for binding of MERS-CoV spike protein (Bosch *et al.*, 2013; van Doremalen *et al.*, 2014; Vergara-Alert *et al.*, 2017). Moreover, primary

horse cells have shown production of infectious virus progeny and viral RNA, which is needed for virus transmission (Bosch *et al.*, 2013; van Doremalen *et al.*, 2014; Vergara-Alert *et al.*, 2017).

Equine Herpesvirustype 1 and 4 (AHV-1 and EHV-4) belong to the subfamily *Alpha-herpesvirinae*, genus *Varicellovirus* of the *Herpesviridae* family. Five strains can infect horses. However, EHV-1 and EHV-4 have the most impact on equine industry (Davison *et al.*, 2009). Both viruses are highly contagious and spread from horse to horse by inhalation of respiratory secretions, as well as direct contact. Latency and periodical reactivation of EHV are responsible for its spread in horses and are key features in the epidemiology of the virus (Roizman, 1996; Slater, 2007; Reed and Toribio, 2004). EHV-1 has a wider host range than that of EHV-4 and can result in neonatal death, abortions, respiratory disease and Equine Herpes virus Myeloencephalopathy (EHM) (Ma *et al.*, 2013). EHV-1 and EHV-4 are different also in virus entry and in their interference with the innate immune response (Patel and Heldens, 2005; Allen *et al.*, 2004). EHV-1 shows a strong tropism for epithelial cells of the upper respiratory tract and affected

horses may have respiratory signs and fever but subclinical infections are common. The virus spreads systematically through infected mononuclear cells to central nervous system or the pregnant uterus leading to neurological disease or abortion (Patel and Heldens, 2005; Allen *et al.*, 2004).

Although equine herpesviruses have worldwide distribution, they did not receive much concern in literature in Saudi Arabia (SA). The only available Virological survey of EHV-1 among horses conducted approximately 15 years ago (Frölich *et al.*, 2005). This study conducted in Najran area, southwest of SA for two main objectives. The first one was to determine the prevalence of infection with equine herpesviruses 1 and 4; and the second was to investigate MERS-CoV infection situation in horses.

Materials and Methods

Study Area and Samples

The study included seven stables in different parts of Najran area. Blood samples collected from 134 horses in risk areas (in close contact with camels). The horses were of different ages and sex and all horses were apparently healthy. After collection, blood samples subdivided into two parts, first part on EDTA for viral RNA detection and the other part without EDTA for MERS-CoV specific antibodies detection. The samples transported to the laboratory in cool boxes. After blood clotting, it was centrifuged at 5,000 rpm for 5 min and sera were separated and stored in labeled cryovials at 20°C till used. Ethical rules and animal welfare were taken into account during sampling. The study approved by the Institutional Ethics Committee at Najran University (ethical number: 15-01-18 EC).

Serological Identification by ELISA

For MERS-CoV identification, the test was done according to the protocol of Meyer *et al.* (2015). The specific S1 antibodies in the serum samples were tested as described by the producer, with slight modification, in which anti-human conjugate were replaced by anti-equine conjugate. For equine herpesviruses identification, the test was done according to the protocol of Lang *et al.* (2013). ELISA kits were purchased from Euroimmune Co, Germany. The optical density was measured by spectrophotometer at 450 nm (TriStar LB941, Berthold).

Positive and negative controls were used in each ELISA performed throughout the study.

Molecular Identification by PCR

For MERS-CoV identification, viral RNA was extracted from blood cells by using DNA-RNA extraction kits (Strattec Molecular, Germany). The RNA extracts were detected by real time quantitative reverse transcriptase PCR (RT-qPCR) by using OneStep RT-PCR reagents according to the manufacturer's instructions (Bioline, Germany). The following primers were used, forward primer: GCAACGCGCGATTTCAGTT, the reverse primer: CCTCTACACGGGACCCATA and the probe: 6FAM-CTCTTACATAATCGCCCCGAGCTCG-BHQ-1.

For herpes virus detection, viral DNA was extracted from the whole blood using the Mini Kit (Strattec Molecular, Germany) from samples according to the manufacturer's instructions. The viral extracts were detected by Real Time Polymerase Chain Reaction (RT-PCR). The protocol was applied according to Damiani *et al.* (2014). Specific primers for EHV-1: (Fw: 50-ATACGTCCCTGTCCGACAGAT-30 and Rv: 50-GGTACT CGGCCTTTGACGAA -30) and a probe (50-6Fam-TGAGACC-GAAGATCTCTCCACCGA-BHQ1-30) targeting EHV-1 gB were used. The same protocol was used for identification of EHV-4 except for using of the following primers and probe: FW 5'-CGC AGA GGA TGG AGA CTT TTA CA -3' RV 5'-CAT GAC CGT GGG GGT TCA A -3' Probe EHV4 5'FAM-CTG CCC GCC GCC TAC TGG ATC-TAMRA. PCR was carried out in 30 cycles of 94°C for 30 sec, 54°C for 30 sec, 72°C for 1 min followed by a 10 min extension at 72°C. PCR was performed with the Applied Biosystems 7500 FAST real-time PCR system.

Results

Anti-MERS-CoV IgG ELISA results showed that all 134 samples were negative for MERS-CoV antibodies. While out of the 134 tested samples for herpesviruses, 31 samples were positive for EHV-1 and 95 samples were positive for EHV-4 (Table 1). The antibodies were detected in all ages and in both males and females. The RT-PCR result was negative for MERS-CoV in the all tested samples. Furthermore, the RT-PCR result showed that all samples of both EHV-1 and EHV-4 were negative.

Table 1: Results of MERS and equine herpes viruses by ELISA and RT-PCR

Virus	Total number of samples	Number of ELISA positive (%)	Number of RT-PCR positive (%)
MERS	134	0 (0%)	0 (0%)
EHV -1	134	31 (23.1%)	0 (0%)
EHV -4	134	95 (70.8%)	0 (0%)

Discussion

World Health Organization (2019) identified MERS-CoV as a potential cause of future epidemics and listed the virus for urgent research. EHV-1 and EHV-4 affect horse populations on all continents. Accurate diagnosis remains a problem facing horse owners and veterinarians (Davison *et al.*, 2009; Roizman, 1996; Slater, 2007). In this study, we examined for the first time the prevalence of these viruses in horses in Najran area. MERS-CoV–endemic areas, such as Saudi Arabia, should be scanned regularly for virus epidemiology. Horses were the target of many studies to examine the possibility of contracting MERS-CoV for several reasons. First, the great similarity between the virus receptors found on both human and horse cells. Second, horses were recently diagnosed with corona virus infection and equine CoV belongs to the same subfamily, Beta-coronavirus, of MERS-CoV. Najran area showed many infections of human and camels with MERS-CoV (Al-Ayed *et al.*, 2014; Alghamdi *et al.*, 2014). Because there is a wide cross contact between camel, horses and human, it was important to identify whether the horses in the region may contract the disease. Our results showed that all samples were negative for MERS antibodies and RNA. Our results are in agreement with other studies that have been done in and out Saudi Arabia (Adney *et al.*, 2016; Hemida *et al.*, 2013; Meyer *et al.*, 2015; Kasem *et al.*, 2018). However, very recent studies showed the ability of the virus to infect ruminant and donkeys (Kandeil *et al.*, 2019; Ali *et al.*, 2017). Further works are needed to follow up this condition.

ELISA results of equine herpesviruses 1 and 4 were determined as high as 22.9 and 70.8% respectively. These results are in accordance with previous studies conducted elsewhere in the world (Kasem *et al.*, 2018; Kandeil *et al.*, 2019; Ali *et al.*, 2017). The viral DNA could not be detected in all ELISA positive samples. The history indicates that these horses were not previously vaccinated or infected, which refer to possible previous subclinical infection with subsequent state of latency. Latent EHV-1 and EHV-4 are widespread in the equines and the site of latency is mainly the lymph nodes of the respiratory tract (Edington *et al.*, 1994; Pusterla *et al.*, 2010; Welch *et al.*, 1992). The seriousness of these results lies in two points. The possibility of reactivation of the latent virus with subsequent overt infection, which could cause heavy economic losses. The second point is the possibility of EHV-1 to become a zoonotic disease and infect human being. The potential of EHV-1 to cross species barriers was demonstrated (Wozniakowski and Samorek-Salamonowicz, 2015; Washburne *et al.*, 2018; Trapp *et al.*, 2005). Productive intranasal infection with EHV-1 of other species as hamster and mouse were shown. Herpesviruses that can infect human cell culture theoretically represent zoonotic threats. EHV-

1 was able to infect human epithelial cells and peripheral blood mononuclear cells (McStreet *et al.*, 1992; Laurent *et al.*, 2001). Although there was no clinical infection and the presence of the nucleic acid was not detected, the very high proportion of equine herpesviruses antibodies, especially type 4, should not be neglected. Horses having latent virus, even at low levels, are considered as potentially risk.

In conclusion, we have determined for the first time in Najran region and even in the whole Saudi Arabia the prevalence of equine herpesviruses antibodies in horses. Following up these horses are important and regular vaccinations should be considered for the future prevention of the disease. Horses did not show any infection with MERS-CoV.

Ethical Approval

The study was approved by the Institutional Ethics Committee at Najran University.

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