Lack of Exposure to Equine Infectious Anemia and Equine Viral Arteritis in Horses in Israel

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ABSTRACT

Equine viral arteritis (EVA) and Equine infectious anemia (EIA) are OIE-listed diseases. EIA has never been diagnosed in Israel, whereas eight horses were EVA-seropositive in 2008. Each year horses are imported to Israel from Europe and North America where both viruses were reported in the past. Furthermore, semen is imported from different European countries and from North America. The purpose of this study was to evaluate the seroprevalence of both EIA and EVA in a population of healthy horses in various geographical locations in Israel. Sera samples were collected from 296 healthy horses from 20 farms across Israel. Commercial ELISA kits were used to determine exposure to these viruses. All samples were seronegative to EIA. All but two samples were seronegative to EVA by ELISA. The two seropositive samples were further analyzed using virus neutralization test (VNT) and were found to be negative. The results of this study indicate that, in 2015, both viruses were not prevalent in the Israeli horse population, despite importation from endemic areas. These findings further support the need to maintain the pre-importation tests that are required in order to protect the Israeli horse population from these pathogens.

Keywords: Horse; Equine Infectious Anemia; Equine Viral Arteritis; Serology; Israel.

INTRODUCTION

Equine viral arteritis (EVA) is an acute, contagious disease, which is exclusively limited to equids and is caused by infection with equine arteritis virus (EAV). Exposure to EAV can occur via respiratory or veneral routes, vertically *in utero* and less frequently via fomites and equipment used in artificial insemination. Clinical signs include fever, anorexia, depression, leucopenia, oedema, conjunctivitis, urticaria, abortion and fatal interstitial pneumonia in young foals. However, most infections with EAV result in subclinical or very mild clinical signs. With the exception of persistently infected stallions (10-70%), EAV is usually cleared from infected animals 28 days after the exposure (1). EVA is endemic in many parts of the world, although the seroprevalence of EAV infection varies between countries, breeds and ages (1). Seroprevalence may reach up to 70-90% in adult Standardbreds in the United States, and 53.2-68.2% in Hucul horses in Poland (2). However, other countries, including Iceland and Japan, are apparently free of the virus (1).

Equine infectious anemia (EIA) is an important blood-borne infectious disease of equids caused by equine infectious anemia virus and is transmitted mechanically by blood-feeding insects (3). EIA virus may also be transmitted *in utero* and iatrogenically by blood transfusions or contaminated needles, surgical instruments and dental floats. EIA acute clinical disease is characterized by pyrexia, thrombocytopenia, anemia, rapid weight loss, petechiae in the mucous membranes, edema and abortion. Disease can also be subclinical with no overt clinical signs.

Both EVA and EIA are OIE-listed diseases (4). According to the Israeli Veterinary Services, EIA has never been diagnosed in Israel. Eight horses tested sero-positive

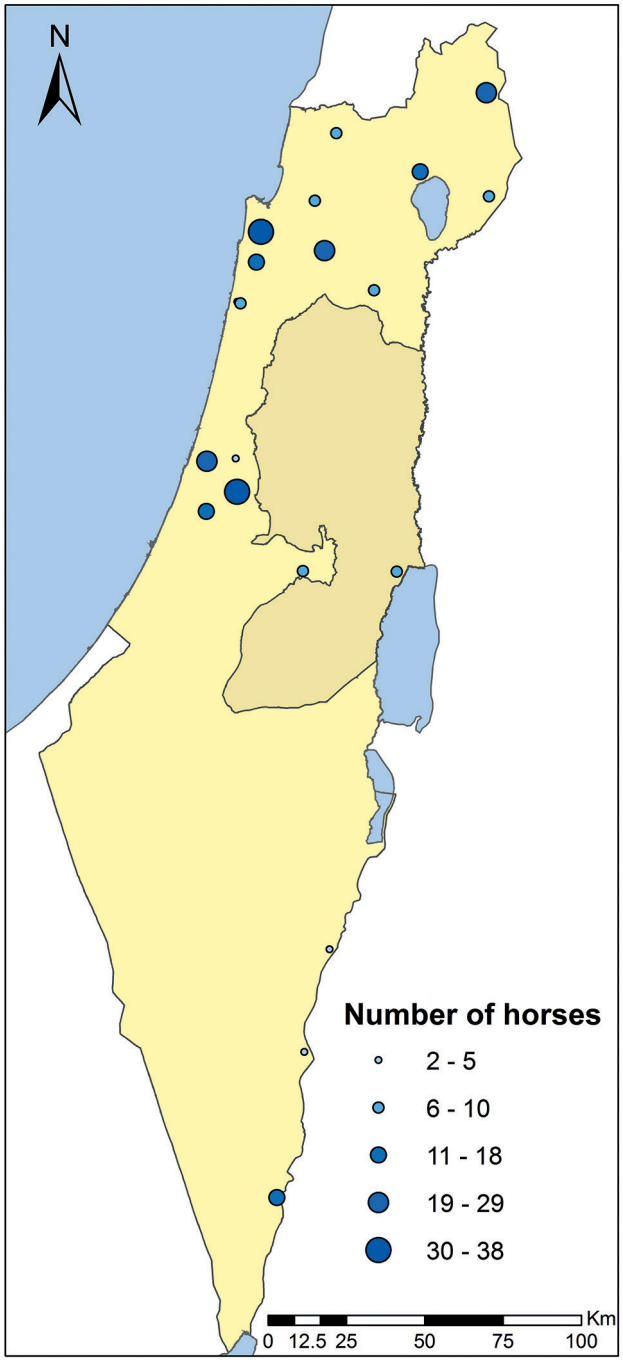

Figure 1: Geographic distribution of the farms sampled. The number of sera samples collected in each farm is represented by the size and color of each circle.

to EVA out of 215 samples tested during an outbreak of febrile disease in horses in Israel during 2008 (5), which was later diagnosed as Equine Encephalosis virus (6). Since then, dozens of samples have been serologically tested every year for EVA and were all negative (7).

Each year horses and horse-semen are imported to Israel from Europe and North America where both viruses have been reported in the past. The prevalence of these two important equine pathogens have never evaluated in Israel. The aim of this study was to evaluate the seroprevalence of EIA and EVA in a population of healthy horses in various geographical locations in Israel.

MATERIALS AND METHODS

Study population – Sera samples were collected from 296 healthy horses in 20 farms across Israel (Fig. 1). Sample size (n=282) for random selection was calculated at 95% confidence interval (CI) and 5% desired absolute precision (DOP) for an expected disease prevalence of 17% (8). Samples were collected from horses that were born in Israel and were never vaccinated against these pathogens.

Blood collections were performed with the owners' consent, and the study was approved by the Internal Ethics Review Committee of the Koret School of Veterinary Medicine, Veterinary Teaching Hospital.

Serological detection of anti-EIA antibodies

A commercial serological ELISA screening kit (ID Screen Equine Infectious Anemia Double Antigen; ID Vet Innovative Diagnostics, Montpellier, France) was used to detect antibodies against the core protein (p26) antigen of EIA virus according to the manufacturer's instructions. This diagnostic kit makes use of the presence of two fragment antibodies (Fab) on IgG or ten Fab on IgM. A first Fab binds the immunoglobulins to the microplate and the other Fab binds a peroxidase antigen used as conjugate. This kit was reported to have a sensitivity of 100% and a specificity of 99.3% (8). Interpretation was performed by calculating the sample to positive ratio (S/P) percentage as recommended by the manufacturer. Agar gel immunodiffusion (AGID) test is considered the gold standard for EIA diagnosis, with sensitivity and specificity of 100% (9).

Serological detection of anti-EAV antibodies

A commercial ELISA kit (ID Screen Equine Viral Arteritis Indirect; ID Vet Innovative Diagnostics, Montpellier, France) was used to detect antibodies directed against EAV, according to the manufacturer's instructions. This kit has been reported to have a sensitivity of 100% and a specificity of

99.38% (8). Interpretation was performed by calculating the S/P percentage as recommended by the manufacturer. The virus isolation (VI) and virus neutralization test (VNT) are the current World Organization for Animal Health (OIE) prescribed standard tests for EAV (10).

Verification of seropositive results using virus neutralization test – Samples suspected positive based on the ELISA were retested using VNT.

RESULTS

The study population consisted of 296 horses from 20 farms and included 154 males (52.1% - 11 stallions and 143 geldings) and 142 females (47.9%). The mean age was 10.87 (±5.4 standard deviation (SDV)) years. Most of the horses (193, 65.2%) were mixed breed horses, and the rest were Quarter horses (49, 16.6%), Tennessee Walking horses (10, 3.4%), Warmbloods (9, 3.0%), Ponies (8, 2.7%), Paint horses (8, 2.7%), Arabians (7, 2.4%), and others (12, 4.1%).

All sera samples were seronegative to EIA. Two samples tested seropositive to EVA using ELISA, but were not confirmed by VNT.

DISCUSSION

The results of this study indicate that, in 2015, both viruses were not prevalent in Israel and although horses were imported to Israel from countries where these diseases occur, carrier horses were probably not imported. These findings further support the need to maintain the pre-importation tests that are required in order to protect the Israeli horse population from these pathogens.

Those pre-importation tests include the following: For horses that are imported from the United States of America it is required that a blood sample was taken within 30 days prior to embarkation, and sent to a laboratory approved by the U.S. Department of Agriculture (USDA), and was found negative to both EIA (Coggins test) and EVA. For horses, that are imported from Europe, it is required that a blood sample was taken within 30 days prior to embarkation. The samples need either to be sent to a laboratory approved by the State Veterinary Services, and found negative to EIA (Coggins test) and negative at a dilution of 1 in 4 to EVA (SN). Or mares and geldings were kept in an establishment where no animals have shown any signs of EVA for the 28 days prior to shipment. Furthermore blood samples were subjected to a test for EVA, carried out on blood samples collected on two occasions at least 14 days apart within 30 days prior to shipment, which demonstrated stable or declining antibody titers; or were regularly vaccinated according to the manufacturer's instructions. For importation of frozen equine semen the stallion should have passed, within 30 days prior to semen collection, a negative immunodiffusion test for EIA. The stallion should also have passed, within 14 days prior to semen collection, a negative EVA test, or at least 0.1 ml of each ejaculate intended for export was tested for EVA (virus isolation) with negative results.

It was previously suggested that the diagnostic assays that are currently approved by the OIE should be replaced with more contemporary assays, such as real-time reverse transcriptase-polymerase chain reaction (rRT-PCR). This test has significant advantages in terms of assay standardization between laboratories, as well as in terms of operational time and costs, and should be considered equivalent or an alternative to Virus Isolation for EAV detection in samples of raw semen (11). These authors also suggested that there is a need to develop highly specific and sensitive ELISA or microsphere immunoassay (Luminex) for the detection of EAV antibodies in serum samples (11). These changes should be made cautiously in order to prevent false negative results and as a result the introduction of pathogens to countries where they were not prevalent before.

Serum samples were first screened through ELISA and then, the two positive samples for EAV, were confirmed by the OIE recommended VNT and were found negative. ELISA is relatively quick and easy to perform but may yield false positive results, which require confirmation by OIE recommended assays as was done in this case. As was previously suggested, the importance of EIA and risk for introduction in disease free countries requires continuous surveillance to maintain the local equine population protected from this disease (12).

CONCLUSIONS

The results of this study indicate that in 2015, EAV and EIA viruses were not prevalent in Israel and although horses and semen were imported to Israel from countries where these diseases occur, carrier horses or infected semen were probably not imported. These findings further support the need to maintain the pre-importation tests that are required in order to protect the Israeli horse population from these pathogens.

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