

## The Serological Investigation of Equine Viral Arteritis Infection in Central Anatolia of Turkey

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**Abstract:** The 380 sera samples were collected from horses in the Konya (208 samples), Kayseri (88 samples) and Ankara (84 samples) regions of Turkey and tested by Enzyme Linked Immunosorbent Assay (ELISA) for the presence of antibodies specific to Equine Arteritis Virus (EAV). Seroprevalance of EAV in the Central Anatolia region of Turkey was 23.4%.

**Key words:** Horse, equine arteritis virus, ELISA, seroprevalance, sera, Turkey

### INTRODUCTION

Equine Arteritis Virus (EAV) is the etiological agent of Equine Viral Arteritis (EVA), a respiratory and reproductive disease that affects horses throughout the world (Weiland *et al.*, 2000). Mortality is rare except in old, young and debilitated horses but economic losses can be significant. The economic impact includes decreased demand for carrier stallions as breeders, deaths in young foals and abortions in 10-60% of susceptible mares. EAV is restricted to the Equidae. Antibodies to this virus have been found in horses, ponies and zebras and outbreaks have occurred in horses and ponies (Timoney *et al.*, 1996; Glaser *et al.*, 1997).

Transmission of EAV occurs via the respiratory route through direct contact with infectious aerosolized nasopharyngeal secretions from acutely infected horses or other secretions such as semen, urine, faeces, vaginal fluids, foetal, placenta and amniotic materials aborted by mares as a result of EAV infection (Timoney and McCollum, 1993). The respiratory route is the primary means of transmission during outbreaks at racetracks, horse shows, sales and veterinary clinics (ISBAH, 2009). The close proximity of probably facilitates the spread of infection by the respiratory route (Monreal *et al.*, 1995). In addition infected stallions play an important role in the epidemiology of the infection as the virus can be transmitted very efficiently by the venereal route (Cole *et al.*, 1986; Del Piero *et al.*, 1997; Glaser *et al.*, 1997).

Laboratory diagnosis of EAV is by virus isolation or serology. Early passage Rabbit Kidney (RK-13) epithelial

cells are the cell-line of choice for virus isolation. Serologic tests include Virus Neutralization, Complement Fixation, Agar Gel Immunodiffusion Indirect Fluorescent Antibody and Enzyme-Linked Immunosorbent (ELISA) Assays (Gilbert *et al.*, 1997; Newton *et al.*, 1999; Guthrie *et al.*, 2003; Szeredi *et al.*, 2003).

The EVA status of most of the horse population in Turkey is unknown as surveillance is not carried out. In this study, serum samples were collected from privately owned horses and the prevalence of EVA antibodies determined in horses in the Central Anatolia region of Turkey.

### MATERIALS AND METHODS

Random sampling points were selected in three provinces in the Central Anatolia region. Blood samples were collected from 380 horses >3 years who did not have any clinical signs of disease (Table 1). The samples were placed in clot activator vacuum tubes and centrifuged at 3000 rpm for 10 min. The separated serum samples were heat-inactivated at 56°C for 30 min before testing. Commercial ELISA kits were obtained from Ingenasa (Spain) and the test was carried out according to the manufacturer's instructions. The results were evaluated spectrophotometrically at 450 nm adsorbance.

Table 1: Positive and negative results of sampled animals and regions

Results	Konya (%)	Kayseri (%)	Ankara (%)	Total (%)
Positive	19.2 (40)	34 (30)	22.6 (19)	23.4 (89)
Negative	80.8 (168)	66 (58)	77.4 (65)	76.6 (291)
Total	208	88	84	380

## RESULTS AND DISCUSSION

Horse samples were collected from the Konya (208 samples), Kayseri (88 samples) and Ankara (84 samples) regions of Turkey and 89 (23.4%) samples were seropositive. The 40 out of 208 samples (19.2%) from the Konya region, 30 out of 88 samples (34%) from the Kayseri region and 19 out of 84 samples (22.6%) from the Ankara region were found as positive by ELISA (Table 1).

In the Central Anatolia region of Turkey, the seroprevalence of EAV has not been reported before. A 23.4% seroprevalence in Central Anatolia is high compared to Kimizigul who reported a seroprevalence of 8.75% in the Kars and Ardahan provinces of Turkey. Vaccine against EAV is not available in Turkey thus all titers in locally bred horses must be due to natural infection.

The higher prevalence of EAV in Central Anatolia may be due to various reasons; improved diagnostic capabilities of laboratories, sampling of horses >3 years old as the prevalence of EAV is higher in older horses (Moraillon and Moraillon, 1978; Paweska *et al.*, 1997). Venereal transmission should be considered in this region since imported carrier stallions may infect the whole region through EAV-infected semen especially if a disease-control programme is not in place. Venereal transmission by a carrier stallion represents the major route of infection for EAV while lateral aerosol spread is a minor route of infection, except in circumstances where clinical respiratory disease or abortions provide a substantial source of virus in an aerosol and prolonged close contact occurs. Aerosol transmission can also occur in cases of acute subclinical infection (Huntington *et al.*, 1990).

A national control strategy for EAV needs to be developed urgently. It is recommended that all potential purchasers of stallions from other countries should establish the EAV serological status of these animals before they are purchased and imported (Newton *et al.*, 1999). In Turkey however, the importation of seropositive horses is not controlled and ignored frequently. Quarantine measures should be put in place to detect carrier stallions before they are imported to Turkey. Husbandry practices which minimize contact between horses and improved standards for transport vehicles especially when transporting recently served mares are other important measure to prevent the spread of EAV and other infectious agents.

Although, some preventative measures are recommended for the control of the EAV infection, these were not carried out by the veterinarians or the breeders in Turkey. Some countries claim to be free of this infection and require serological testing of horses before importation is permitted as does the European Union (EU). It is recommended that all potential purchasers of stallions

from other countries should establish the EVA serological status of these animals before they are purchased and imported (Newton *et al.*, 1999). In Turkey however, the importation of seropositive horses is not controlled as it should be and ignored frequently. Therefore, the control strategy should be developed urgently since, the seroprevalence of EAV infection is 23.4% in Turkey.

## CONCLUSION

This study shows that preventative measures could include continued quarantine strategies designed to detect carrier stallions before they are imported to Turkey and horse husbandry practices which minimize contact between horses of other breeds standards for transport vehicles especially when transporting recently served mares is another important measure to prevent spread of EAV and other infectious agents.

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## REFERENCES

- Cole, J.R., R.F. Hall, H.S. Gosser, J.B. Hendricks and A.R. Pursell *et al.*, 1986. Transmissibility and abortogenic effect of equine viral arteritis in mares. *J. Am. Vet. Med. Assoc.*, 189: 769-771.
- Del Piero, F., P.A. Wilkins, J.W. Lopez, A.L. Glaser, E.J. Dubovi, D.H. Schlafer and D.H. Lein, 1997. Equine viral arteritis in newborn foals: clinical, pathological, serological, microbiological and immunohistochemical observations. *Equine Vet. J.*, 29: 178-185.
- Gilbert, S.A., P.J. Timoney, W.H. McCollum and D. Deregt, 1997. Detection of equine arteritis virus in the semen of carrier stallions by using a sensitive nested PCR assay. *J. Clin. Microbiol.*, 35: 2181-2183.
- Glaser, A.L., E.D. Chimside, M.C. Horzinek and A.A.F. De Vries, 1997. Equine arteritis virus. *Theriogenology*, 47: 1275-1295.
- Guthrie, J., P.G. Howell, J.F. Hedges, A.M. Bosman and U.B.R. Balasuriya *et al.*, 2003. Lateral transmission of equine arteritis virus among Lipizzaner stallions in South Africa. *Equine Vet. J.*, 35: 596-600.
- Huntington, P.J., A.J. Forman and P.M. Ellis, 1990. The occurrence of equine arteritis virus in Australia. *Aust. Vet. J.*, 67: 432-435.
- ISBAH, 2009. Equine viral arteritis. Indiana State Board of Animal Health Technical Bulletin EQ-411.99. <http://www.in.gov/boah/files/cp-4899.pdf>.

- Monreal, L., A.J. Villatoro, H. Hooghuis, I. Ros and P.J. Timoney, 1995. Clinical features of the 1992 outbreak of equine viral arteritis in Spain. *Equine Vet. J.*, 27: 301-304.
- Moraillon, R. and A. Moraillon, 1978. Results of a serological survey of viral arteritis in France and several European and African countries. *Ann. Vet. Res.*, 9: 43-54.
- Newton, J.R., J.L.N. Wood, F.J. Castillo-Olivares and J.A. Mumford, 1999. Serological surveillance of equine viral arteritis in the United Kingdom since the outbreak in 1993. *Vet. Rec.*, 30: 511-516.
- Paweska, J.T., M.M. Binns, P.S. Woods and E.D. Chirnside, 1997. A survey for antibodies to equine arteritis virus in donkeys, mules and zebra using Virus Neutralization (VN) and Enzyme Linked Immunosorbent Assay (ELISA). *Equine Vet. J.*, 29: 40-43.
- Szeredi, L., A. Hornyak, B. De'nes and M. Rusvai, 2003. Equine viral arteritis in a newborn foal: Parallel detection of the virus by immunohistochemistry, polymerase chain reaction and virus isolation. *J. Vet. Med. B*, 50: 270-274.
- Timoney, P.J. and W.H. McCollum, 1993. Equine viral arteritis. *Vet. Clin. N. Am. Food A*, 9: 295-309.
- Timoney, P.J., B. Klingeborn and M.H. Lucas, 1996. A perspective on equine viral arteritis (infectious arteritis of horses). *Rev. Sci. Tech.*, 15: 1203-1208.
- Weiland, E., S. Bolz, F. Weiland, W. Herbst, M.J. Ramsman, P.J. Rottier and A.A. De Vries, 2000. Monoclonal antibodies directed against conserved epitopes on the nucleocapsid protein and the major envelope glycoprotein of equine arteritis virus. *J. Clin. Microbiol.*, 38: 2065-2075.