

## Seroprevalence of Equine Viral Arteritis in Donkeys in Kars District, Turkey

<sup>1</sup>Yakup Yildirim, <sup>2</sup>Ali Haydar Kirmizigul, <sup>3</sup>Mehmet Tolga Tan,

<sup>2</sup>Erhan Gokce and <sup>2</sup>Kemal Irmak

<sup>1</sup>Department of Virology, <sup>2</sup>Department of Internal Medicine,

Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

<sup>3</sup>Department of Virology, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın, Turkey

**Abstract:** This study investigated sero-prevalence of Equine Viral Arteritis (EVA) in Kars district. For this purpose, 76 donkeys from the district were blood sampled and specific antibodies to equine viral arteritis were investigated in serum by means of Enzyme Linked Immunosorbent Assay (ELISA). Of the population sampled, seroprevalence of EVA was 14.47% (11/76). Seropositivity according to gender revealed that the proportion of seropositivity was 16.66% (9/54) in male donkeys and 9.09% (2/22) in female donkeys. This was the first study to report the presence and sero-prevalence of EVA in donkeys in Northeast Anatolia where donkey has important contribution to economic and day to day life of human and preventive measures should be taken to control the infection.

**Key words:** Donkey, ELISA, equine viral arteritis, seroprevalence

### INTRODUCTION

Equine Viral Arteritis (EVA) is reproductive and respiratory disease of horses, donkey and mules that is caused by equine arteritis virus (EAV) (Balasuriya *et al.*, 2004). EAV is small positive-stranded RNA virus that was recently placed in the family Arteriviridae (genus Arterivirus) in the order Nidovirales (Hedges *et al.*, 1998).

EAV was first detected from lung tissue during an outbreak of respiratory disease and abortion in Standard-bred horses in the United States in 1953 (Paweska *et al.*, 1995). Serological and clinical evidence indicate that EAV has a worldwide distribution and seroreactivity has been reported in horses, donkeys, mules and zebras (Glaser *et al.*, 1997). Only one serotype of EAV has been demonstrated although strains of EAV have been shown to differ in their pathogenicities (Paweska *et al.*, 1995).

The causal agent of EAV is mainly transmitted either via nasal and eye discharges and urine of animals aborted or acutely infected animals. In addition, the semen of infected stallion and male donkeys may also play an important role in virus dissemination. Persistent infections may be developed in stallion and male donkeys that serve as natural reservoir of the virus (Timoney and McCollum, 2000, 1996; Paweska *et al.*, 1995).

The clinical signs of EVA infection can vary from subclinical to severe disease and death. Each individual may not show all the same signs (Hedges *et al.*, 1998;

Balasuriya *et al.*, 2004). EAV infection of horses is endemic in most parts of the world (Balasuriya *et al.*, 2004). While, the majority of EAV infections of horses are typically subclinical or asymptomatic, acutely infected animals may develop a wide range of clinical signs, including pyrexia, congestion, oedema in limbs and genital organ, diarrhea, hemorrhagic enteritis, depression, rhinitis, dyspnea, laryngitis, pharyngitis, coughing and conjunctivitis (Glaser *et al.*, 1997; Wagner *et al.*, 2003; Del Piero, 2000; Chirnside, 1993; Moore *et al.*, 2003; Timoney and McCollum, 1996). The virus may cause abortion and also mortality in neonates (Samper and Tibary, 2006; Glaser *et al.*, 1997). Clinical form in the donkey of the EAV infection is similar to that of horses (Paweska *et al.*, 1995, 1996).

Clinically, EAV resembles several other viral infections of equines, so that a definitive diagnosis requires laboratory confirmation (Glaser *et al.*, 1997). Several methods such as Enzyme Linked Immunosorbent assay (ELISA) (Paweska *et al.*, 1997; Cho *et al.*, 2000), Polymerase Chain Reaction (PCR) (Ramina *et al.*, 1999) and Serum Neutralisation (SN) (Cho *et al.*, 2000; Paweska *et al.*, 1997) can be used to diagnose the EVA infection.

The ELISA is sensitive and specific for the detection of EAV-specific antibodies in horse, donkey and mule sera and can detect the presence of specific antibodies the antibody titers can correlate well. The ELISA can also

detect differences between IgG and IgM levels in donkeys that is important to distinguish between recent and previous EAV infections. Another ELISA based on the GL protein has been developed which used multiple ectodomain sequences from phylogenetically different isolates. An ELISA based on the N protein is also highly sensitive and specific and may allow differentiation between the antibody response promoted by a subunit vaccine from that of infection (Glaser *et al.*, 1997).

In comparative studies using ELISA and SN tests for evaluation of EAV specific antibodies in the blood serum, it was found that the sensitivity was 99.4-99.2% (Cho *et al.*, 2000) and specificity was 80.3% (Paweska *et al.*, 1997). In addition, ELISA is less time consuming and cheaper than SN (Cho *et al.*, 2000).

In this study, the presence of EAV infections in donkeys in Kars province was detected serologically for the first time.

## MATERIALS AND METHODS

**Serum samples:** In this study, blood serum samples were collected randomly from 76 unvaccinated donkeys (54 male and 22 female) older than 1 year in Kars province. The serum samples were inactivated at 56°C for 30 min and were stored at -20°C until use.

**Enzyme Linked Immunosorbent Assay (ELISA):** For detection of EAV antibodies a commercial indirect ELISA (Ingenasa-Ispanya) as used. Test as performed according to the manufacturer's directions. The results were evaluated by reading of plates in 450 nm spectrophotometer at the final step.

**Statistical analysis:** Chi-square ( $\chi^2$ ) method was used to compare the proportion of positivity to EAV infection between male and female (SPSS, 1999). Significant level was set of  $p < 0.005$ .

## RESULTS

The seropositivity of EAV in the blood serum samples from 76 donkeys by ELISA was found as 14.47% (11/76). The proportions of seropositive male and female donkeys were 16.66% (9/54) and 9.09% (2/22), respectively. This difference was not statistically significant (OR = 2.0-95% CI; 0.35-14.8,  $\chi^2 = 0.24$ ,  $p = 0.6$ ).

## DISCUSSION

Due to the heavy winter conditions and a wide range of uneven lands in Kars district, horses, donkeys and mules still serve an important role in human life

participating in transport and agriculture. EVA is manifested by abortions and other disorder in this species leading to economical losses.

Studies carried out in donkeys and horses in other countries, the seroprevalence of EVA ranged from 1.9 to 18.6% in horses (Szeredi *et al.*, 2005; Hullinger *et al.*, 2001; Ghram *et al.*, 1994; Paweska *et al.*, 1995; Paweska *et al.*, 1996; Paweska *et al.*, 1997). The presence of EVA infections of horses in Turkey was previously reported by Yılmaz *et al.* (1996). Study by Kırmızıgül *et al.* (2007) reported the seroprevalence of EVA infections in horses as 8.75% Kars and Ardahan province of Turkey.

Ramina *et al.* (1999) showed the the presence of EVA in horse and donkey semen by using PCR. The EVA virus can cause persistent infections in male donkeys that release the virus with their semen. Thus, these animals was be considered main virus reservoirs (Paweska *et al.*, 1995, 1996, 1997; Paweska and Barnard, 1993).

Our study is the first to determine the serological status of EVA infection in donkeys in northeast region of Turkey. The seroprevalence of EVA was 14.47% and the seropositivity rate was 16.66% in male donkeys and 9.09% in female donkey. Although, the seropositivity rate of male donkeys was higher than that of female, this was not statistically significant. A similar study carried out in horses in Kars region the seropositivity was 9.5%. The implies that EVA infection is present in equides in the region (Kırmızıgül *et al.*, 2007).

## CONCLUSION

This was the first study to report the prevalence of EAV in donkeys in Northeast Anatolia where donkey has important contribution to economic and day to day life of human and preventive measures (prophylactic vaccine, routinely control for EVA viruses of the male vb) should be taken to control the infection. In addition, it is important to stop uncontrolled animal movements between north east part of Turkey and neighbouring countries.

## REFERENCES

- Balasuriya, U.B.R., J.F. Hedges and V.L. Smalley *et al.*, 2004. Genetic characterization of equine arteritis virus during persistent infection of stallions. *J. General Virol.*, 85: 379-390.
- Chimside, E.D., 1993. Equine viral arteritis: A free market threat. *Eq. Vet. Edu.*, 5: 137-139.
- Cho, H.J., S.C. Entz, D. Deregt, L.T. Jordan, P.J. Timoney and W.H. McCollum, 2000. Detection of antibodies to equine arteritis virus by a monoclonal antibody based blocking ELISA. *Can. J. Vet. Res.*, 64: 38-43.

- Del Piero, F., 2000. Equine viral arteritis. *Vet. Pathol.*, 37: 287-296.
- Ghram, A., A. Chabchoub, I. Turki, M. Boussetta, H. Ibn Amor and A. Ghorbel, 1994. Rhinopneumonia and equine viral arteritis: Seroepidemiological study in the northeast of Tunisia. *Arch. Inst. Pasteur Tunis*, 71: 5-12.
- Glaser, A.L., E.D. Chirnside, M.C. Horzinek and A.A.F. Vries, 1997. Equine arteritis virus. *Theriogenol.*, 47: 1275-1295.
- Hedges, J.F., U.B.R. Balasuriya, S. Ahmad, P.J. Timoney, W.H. McCollum, T. Yilma and N.J. MacLachlan, 1998. Detection of antibodies to equine arteritis virus by enzyme-linked immunosorbent assays utilizing GLI M and N proteins expressed from recombinant baculoviruses. *J. Virol. Methods*, 76: 127-137.
- Hullinger, P.J., I.A. Gardner, S.K. Hietala, G.L. Ferraro and N.J. MacLachlan, 2001. Seroprevalence of antibodies against equine arteritis virus in horses residing in the United States and imported horses. *J. Am. Vet. Med. Assoc.*, 219: 946-949.
- Kırmızıgül, A.H., Y. Yıldırım and G. Gökçe, 2007. Kars ve Ardahan Yöresindeki Atlarda Equine Viral Arteritis Enfeksiyonunun Seroprevalansının Belirlenmesi. *Kafkas Üniv. Vet. Fak. Derg.*, 13: 171-175.
- Moore, B., U. Balasuriya, J. Watson, C. Bosio, R. MacKay and N. MacLachlan, 2003. Virulent and avirulent strains of equine arteritis virus induce different quantities of TNF-alpha and other proinflammatory cytokines in alveolar and blood-derived equine macrophages. *Virology*, 314: 662-670.
- Paweska, J.T., D.H. Volkmann, B.J.H. Barnard and E.D. Chirnside, 1995. Sexual and In-Contact Transmission of Asinine Strain of Equine arteritis Virus among Donkeys. *J. Clin. Microbiol.*, 33: 3296-3299.
- Paweska, J.T., H. Aitchison, E.D. Chirnside and B.J.H. Barnard, 1996. Transmission of the South African asinine strain of equine arteritis virus (EAV) among horses and between donkeys and horses. *Onderstepoort J. Vet. Res.*, 63: 189-96.
- 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 neutralisation (VN) and enzyme linked immunosorbent assay (ELISA). *Equine Vet. J.*, 29: 40-43.
- Paweska, J.T. and B.J.H. Barnard, 1993. Serological evidence of equine arteritis virus in donkeys in South Africa. *Onderstepoort J. Vet. Res.*, 60: 155-158.
- Timoney, P.J. and W.H. McCollum, 2000. Equine viral arteritis: Further characterization of the carrier state in stallions. *J. Reprod. Fertil. Suppl.*, 56: 3-11.
- Timoney, P.J. and W.H. McCollum, 1996. Equine viral arteritis. *Eq. Vet. Edu.*, 8: 97-100.
- Ramina, A., L. Dalla Vale, S. De Mas, E. Tisato, A. Zuin, M. Renier, V. Cuteri, C. Valente and F.E. Cancellotti, 1999. Detection of equine arteritis virus in semen by reverse transcriptase polymerase chain reaction-ELISA. *Comp. Immun. Microbiol. Infect. Dis.*, 22: 187-197.
- Samper, J.C. and A. Tibary, 2006. Disease transmission in horses. *Theriogenology*, 66: 551-559.
- SPSS for Windows, 1999. SPSS Copyright Ins. Version 10.0.
- Szeredi, L., A. Hornyak, V. Palfi, T. Molnar, R. Glavits and B. Denes, 2005. Study on the epidemiology of equine arteritis virus infection with different diagnostic techniques by investigating 96 cases of equine abortion in Hungary. *Vet. Microbiol.*, 108: 235-242.
- Wagner, H.M., U.B.R. Balasuriya and N.J. MacLachlan, 2003. The serologic response of horses to equine arteritis virus as determined by competitive enzymelinked immunosorbent assays (c-ELISAs) to structural and non-structural viral proteins. *Comp. Immunol. Microbiol. Infect. Dis.*, 26: 251-260.
- Yılmaz, H., N.Y. Özgür and A. Ilgaz, 1996. Equine viral arteritis üzerine serolojik araştırma. I. Uluslararası Veteriner Mikrobiyoloji Kongresi, İstanbul, pp: 200.