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VEYSEL SOYDAL ATASEVEN

JANET MARY DALY

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Seroepidemiology of Equine Influenza Virus Infection in Turkey

Veysel Soydal ATASEVEN^{1,*}, Janet Mary DALY²

¹Department of Virology, Faculty of Veterinary Medicine, Mustafa Kemal University, Tayfur Sökmen Campus, 31040 Antakya, Hatay - TURKEY

²Centre of Preventive Medicine, Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk-UK

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Abstract: The aim of this study was to investigate the seroepidemiology of equine influenza infection in Turkey, measured using the haemagglutination inhibition (HI) assay. Serum samples were obtained from 623 equids aged older than 4 years from 5 different geographical regions in Turkey during the years 2003-2005. Antibodies against equine influenza virus (EIV) were found in 194 (31.1%) of the 623 sera. Seropositivity rates were detected as 41.8% (171/409), 12.8% (11/86), and 9.4% (12/128) in horses, mules, and donkeys, respectively.

The data revealed that EIV circulates in Turkey at a relatively high rate. Differences in seropositivity rates in Equidae from different geographical regions probably reflect regional differences in their economic uses.

Key Words: Equine influenza virus, antibody, donkey, horse, mule, Turkey

Türkiye'de Equine Influenza Virus Enfeksiyonunun Seroepidemiolojisi

Özet: Bu çalışmanın amacı, Türkiye'de equine influenza (EI) enfeksiyonunun sero-epidemiolojisinin hemagglütinasyon inhibisyon (HI) testi ile araştırılmasıdır. 2003-2005 yılları arasında Türkiye'nin 5 farklı coğrafi bölgesinden, 4 yaşının üzerindeki 623 tektirnaklı hayvandan kan örneği alındı. 623 adet kan serumunun 194 adedinde (% 31,1) EI virusa spesifik antikorlar tespit edildi. Seropozitiflik oranları at, katır ve eşeklerde sırasıyla % 41,8 (171/409), % 12,8 (11/86) ve % 9,4 (12/128) olarak belirlendi.

Elde edilen bulgular, EI virus enfeksiyonunun Türkiye'de yüksek düzeyde sirküle olduğunu ortaya koymaktadır. Coğrafi bölgelerdeki seropozitiflik oranlarındaki farklılıklar, tektirnaklıların ekonomik amaçlı kullanımlarındaki bölgesel farklılıkları da yansıtmaktadır.

Anahtar Sözcükler: Equine influenza virus, antikor, eşek, at, katır, Türkiye

Equine influenza is a severe, acute respiratory disease with characteristic clinical signs of pyrexia, dyspnoea, anorexia, and coughing, which is caused by 2 subtypes of influenza A virus (H7N7 and H3N8) of the family *Orthomyxoviridae*. H7N7 viruses have not been isolated for a long time, although there is serological data that the virus may circulate sub-clinically (1), whereas epidemics caused by H3N8 viruses occur frequently worldwide (2). The H3N8 subtype has split into 2 distinct lineages, the American lineage represented by Newmarket/1/93, and the European lineage represented by Newmarket/2/93 (3).

Equids are important for transport and farming in Turkey. Additionally, raising horses for racing is an economically important sector. No studies on the prevalence of equine influenza virus (EIV) infection in Turkey have been reported. The aim of this study was, therefore, to investigate the prevalence of antibodies to EIV in sera from Equidae in different regions of Turkey.

For this purpose, 623 serum samples were randomly taken from horses, mules, and donkeys older than 4 years from 5 different geographical regions in Turkey (Figure) during 2003-2005. The sera were periodate-treated and heat-inactivated to remove non-specific inhibitors of haemagglutination. One volume (150 µl) of serum and 2

* E-mail: soydalata@hotmail.com

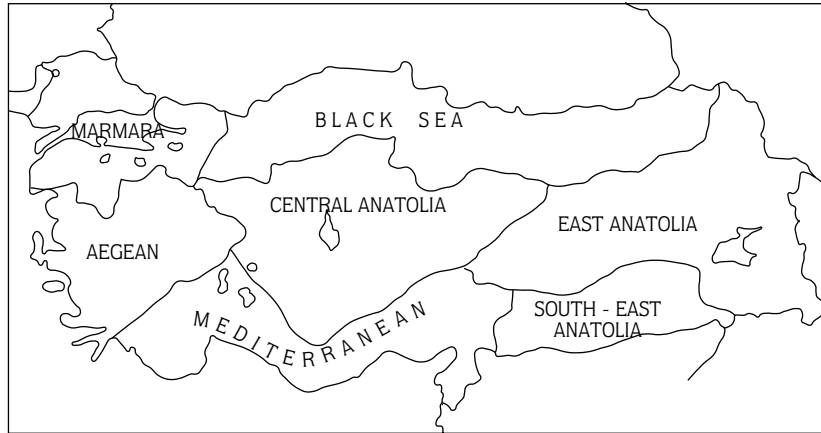


Figure. Map of Turkey showing the 7 different geographical regions.

volumes (300 μ l) of freshly prepared 0.016 M potassium periodate (KIO_4) were mixed at room temperature for 15 min. A further volume of 3% glycerol in phosphate-buffered saline (0.01M, pH 7.2) (v/v) and treated sera were mixed at room temperature for 15 min to neutralise any excess periodate solution. Heat-inactivation was performed in a 56 °C-water bath for 30 min.

The treated sera were tested for the presence of antibodies against EIV as described by Goto et al. (4), using 4 haemagglutination (HA) units of virus. Serial 2-fold dilutions of sera (1:8 to 1:1024) were prepared with phosphate-buffered saline (0.01 M, pH 7.2) in microplates with V-bottoms. Phosphate-buffered saline (25 μ l) was dispensed in all wells. An equal amount of serum was added to the first well of a row of 12 and was titrated. The last well was left as a control. The antigen was diluted to give a dose of 4 HA units. To each serum dilution 25 μ l of 4 HA units of virus were added. All plates were incubated at room temperature for 30 min. To all wells 50 μ l of 1% chicken erythrocytes (RBCs) was added and all the plates were incubated at room temperature for 30 min. The results were read as reciprocals of the highest dilution of serum that completely inhibited haemagglutination.

The strains of EIV used as antigen in the HI assay were A/eq/Newmarket/1/93 (N/1/93; prototype strain for the H3N8 American lineage), A/eq/Newmarket/2/93 (N/2/93; prototype strain for the H3N8 American

lineage), and A/eq/Prague/56 (prototype strain for the H7N7 subtype). Virus strains were treated with Tween 80 and ether before use (4).

Antibodies against EIV were found in 194 (31.1%) of the 623 sera tested and in all geographical regions sampled (Table). In all, 409 horses were sampled, of which 169 (41.3%) were positive for the H3N8 antibody. Of the 86 mules, 11 (12.8%) were positive for H3N8 antibodies, as were 12 of the 128 (9.4%) donkeys. Samples from 29 (4.7%) animals, all of which were horses, were positive for antibodies to the H7N7 subtype of EIV, of which 28 horses were from the Marmara region (2 of which had antibodies against only the H7N7 subtype). The other H7N7-positive sample was from a horse in East Anatolia and this horse also had antibodies to H3N8. No samples from the other 3 regions were seropositive to the H7N7 strain and all samples from donkeys and mules were negative for antibodies to the H7N7 subtype of EIV (Table).

The highest seropositivity rates for EIV infection were detected in the Marmara (60.2%) and East Anatolia (35.6%) regions. In Marmara, horses are bred for racing and riding and there is a large Thoroughbred horse population, as well as importation of horses from outside Turkey. It is likely that the high seropositivity in Marmara and the presence of antibodies to the H7N7 subtype virus are results of vaccination with vaccines containing H7N7 and H3N8 subtypes.

Table. Distribution of sera with HI assay antibodies to the H3N8 viruses (Newmarket/1/93 and/or Newmarket/2/93) and the H7N7 virus, according to region and species.

| Region | Species | Tested sera | Ab to H7N7 Positive (%) | Ab to N/1/93 Positive (%) | Ab to N/2/93 Positive (%) | Ab to H3N8 subtype Positive (%) | Ab to H3N8 and/or H7N7 Positive (%) |
|---------------------|---------|-------------|-------------------------|---------------------------|---------------------------|---------------------------------|-------------------------------------|
| East Anatolia | Horse | 250 | 250 | 1 (31.6) | 44 (17.6) | 93 (37.2) | 93 (37.2) |
| | Mule | 16 | – | 6 (37.5) | 3 (18.8) | 6 (37.5) | 6 (37.5) |
| | Donkey | 37 | – | 8 (21.6) | 4 (10.8) | 9 (24.3) | 9 (24.3) |
| | Total: | 303 | 1 (0.3) | 93 (30.7) | 51 (16.5) | 108 (35.6) | 108 (35.6) |
| South-east Anatolia | Horse | 12 | – | 2 (16.7) | 2 (16.7) | 2 (16.7) | 2 (16.7) |
| | Mule | 23 | – | 4 (17.4) | 4 (17.4) | 4 (17.4) | 4 (17.4) |
| | Donkey | 14 | – | 2 (14.3) | 2 (14.3) | 2 (14.3) | 2 (14.3) |
| | Total: | 49 | – | 8 (16.3) | 8 (16.3) | 8 (16.3) | 8 (16.3) |
| Mediterranean | Horse | 32 | – | 6 (18.8) | 6 (18.8) | 6 (18.8) | 6 (18.8) |
| | Mule | 27 | – | – | 1 (3.7) | 1 (3.7) | 1 (3.7) |
| | Donkey | 40 | – | 1 (2.5) | 1 (2.5) | 1 (2.5) | 1 (2.5) |
| | Total: | 99 | – | 7 (7.1) | 2 (2.0) | 8 (8.1) | 1 (2.5) |
| Black Sea | Horse | 2 | – | 2 (100) | – | 2 (100) | 2 (100) |
| | Mule | 20 | – | – | – | 0 | 0 |
| | Donkey | 37 | – | – | – | 0 | 0 |
| | Total: | 59 | – | 2 (3.4) | – | 2 (3.4) | 2 (3.4) |
| Marmara | Horse | 113 | 28 (24.8) | 43 (38.0) | 34 (30.1) | 66 (58.4) | 68 (60.2) |
| | Mule | 0 | – | – | – | 0 | – |
| | Donkey | 0 | – | – | – | 0 | – |
| | Total: | 113 | 28 (24.8) | 43 (38.0) | 34 (30.1) | 66 (58.4) | 68 (60.2) |
| Total | | 623 | 29 (4.7) | 153 (24.3) | 95 (15.3) | 192 (30.8) | 194 (31.1) |

In East Anatolia the seropositivity rates were similar irrespective of the Equidae species (24.3%-37.5%). Additionally, the highest seropositivity rates of EIV infection in mules and donkeys were detected in this region. The inhabitants of East Anatolia rely heavily on Equidae for transport because this region is geographically remote and has a harsh climate. In this region, there are generally 10 to 25 Equidae per household and Equidae are frequently traded. This region is also bordered by Iran, for which the status of EIV infection is still unknown, and frequent Equidae movement, including illicit cross-border trading of animals, may increase the risk of infection in this region.

The Black Sea, Mediterranean, and South-east Anatolia regions may have had lower seropositivity than the Marmara and East Anatolia regions because importation of Equidae, animal movement, and trade are rare, as Equidae are locally used primarily as pack animals in these 3 regions.

Antibodies against the American lineage strain (Newmarket/1/93) predominated and titres were higher (unpublished data), but antibodies to the European lineage strain (Newmarket/2/93) were also found at remarkable levels (Table). Virus isolation and characterisation would be required to make a definitive statement about the strains circulating in Turkey.

The results of this investigation showed that EIV circulates among Equidae in Turkey at a relatively high rate. Further studies should include attempted isolation of the aetiological agent and its characterisation. Epidemiological investigations should shed light on the true status and of equine influenza infection in Turkey

and help to inform control programmes. Additionally, it is suggested that the local authorities in East Anatolia should be made aware of the unofficial Equidae trade and transport between this region and border countries as a potential route of introduction of equine infectious diseases.

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