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A pilot study on the epidemiological status of equine infectious anaemia, equine viral arteritis, glanders, and dourine in Turkey

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Abstract: A serological investigation of equine infectious anaemia (EIA), equine viral arteritis (EAV), glanders, and dourine was conducted on the sera of 346 Turkish horses using a combination of tests in series (ELISA and agar gel immunodiffusion) for EIA, virus neutralisation for EAV, and complement fixation (CF) for glanders and dourine. Forty-nine sera showed anticomplementary reactions and were not assessable in the CF test for glanders and dourine. No positive samples were detected for EIA, dourine, and glanders. Fifty-seven sera were positive for EAV. A systematic review of the distribution of these diseases in Turkey was conducted to describe their epidemiological status in the country. The serological results of this investigation confirm those of the published reports for EAV and glanders, whereas different results were reported for dourine and EIA. In fact, no previous data were found for dourine. Furthermore, all sera tested for EIA in the literature were negative, but 3 outbreaks were reported on an international official site in 2005 without details. Further studies and reports of the outbreaks are needed to better understand the real status of infections and the transmission of the diseases. The systematic review is a useful tool to improve the knowledge of public health disease in a country.

Key words: Equine infectious anaemia, equine viral arteritis, glanders, dourine, Turkey, systematic review

1. Introduction

Due to its geographic significance as a land bridge between Asia and Europe, Turkey is an important region for the control and prevention of the spread of contagious diseases in animals. The equid population is important in Turkey, being estimated at 525,531 units in 2009 (http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalpopulation), and is used for working, racing, and breeding. Exportation and importation movements at the border create a high risk for the equid population, particularly if the status of infections is not known. In this situation, contagious diseases are a very important issue. Equine infectious anaemia (EIA), equine viral arteritis (EAV), glanders, and dourine are listed as reportable diseases by the World Organisation for Animal Health (OIE), as they are considered relevant to international trade (http://www.oie.int/en/animal-health-in-the-world/oie-listed-diseases-2012/).

EIA is limited to equids and occurs worldwide. The causative agent, the EIA virus (EIAV), is a lentivirus of the family *Retroviridae*, subfamily *Orthoretrovirinae*. EIA is characterised by recurrent febrile episodes, thrombocytopenia, anaemia, rapid weight loss, and oedema of the lower parts of the body. If the acute clinical attack does not provoke death, the infected horse can remain a viraemic carrier and potentially transmit the infection to susceptible horses by means of bloodsucking horseflies or iatrogenically. In utero infection of the foetus may also occur (1).

EAV is a worldwide contagious disease caused by the equine arteritis virus (order *Nidovirales,* family *Arteriviridae*, genus *Arterivirus)* that especially affects

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equines (2). Many cases of infections are subclinical, but cases with different severities have been reported (2,3). The typical clinical signs are as follows: depression; high temperature; anorexia; rhinitis; conjunctivitis; ocular and nasal discharge; supra- or peri-orbital oedema; oedema localised in the ventral abdomen, prepuce, scrotum, or legs; local or generalised urticaria; leucopenia; thrombocytopenia; abortion; and stillbirth. Young foals can be severely affected. A variable percentage of infected stallions can excrete the virus in their semen and become persistently infected. This is the main cause of perpetuation of the virus in equine populations. An increase in the incidence of EAV has been observed in recent years due to the increased transfer of horses and the use of frozen semen for artificial insemination (2).

Burkholderia mallei (formerly *Pseudomonas mallei*) is the causal agent of glanders, a contagious disease of horses, mules, and donkeys, that can be transmitted to humans and other mammals. The infection causes typical nodules in the viscera, which can ulcerate, especially in the respiratory tract. When the lesions are localised at skin level, the disease is called 'farcy'. Subclinical cases can occur and they are a serious source of infection because of the possibility of permanent or intermittent spread of the bacteria. Close contact, along with the ingestion or inhalation of infected materials, facilitates transmission among animals (4).

Dourine is caused by *Trypanosoma equiperdum* and is naturally transmitted only by coitus. Dourine is different from the other forms of trypanosomiasis in that no invertebrate vectors or natural reservoirs of the parasite are recognised other than infected equids. The genital secretions of infected females and males discharge the parasite. Chronic and acute forms are described. The incubation period, the severity of the clinical syndrome, and its duration vary depending on the health of the equine population and the involved strain; subclinical infections and unapparent carriers can occur, in particular in mules and donkeys. The disease is characterised by different stages in which severe clinical signs alternate with periodic relapse. The culmination can be death or sometimes recovery. The main clinical signs consist of the following: swelling and oedema of the prepuce, scrotum and mammary glands; fever; cutaneous urticaria; progressive anaemia; weakness; emaciation; paraplegia; and incoordination. The typical oedematous plaques of the skin, 2–5 cm in diameter, are considered pathognomonic lesions for dourine when they are present (5).

The aim of this study was to evaluate the presence of antibodies against EIAV, EAV, *Burkholderia mallei*, and *Trypanosoma equiperdum* in a Turkish horse population and to examine the scientific evidence of these infections in Turkey, in order to evaluate the global risk of the presence of these infections in Turkey and the consequent introduction to neighbouring countries.

2. Materials and methods

2.1. Sample collection

In 2004, serum samples were obtained from 346 horses, including 134 females and 212 males, coming from 19 different stud farms without exchanges or relationship among them. The farms were located in the İstanbul, İzmir, and Bursa provinces, all of which border Europe. Samples were stored at –20 °C until use. The sampling included horses that ranged from 1 month to 10 years of age, with a mean age of 3 years, and the breeds represented were Arabian, Thoroughbred, and mixed-breed.

2.2. Serological tests

The most reliable serological test for each infection was selected. A commercial serological screening kit called ELISA (Kit ELISA ID Screen®, IDVet Innovative diagnostics, Montpellier, France), incorporating both core protein (p26) and viral transmembrane protein (gp45) antigens, was used to detect antibodies against EIAV. Inconclusive and positive sera were tested again using agar gel immunodiffusion (AGID), as indicated for international trade by OIE (1). The combination of these 2 tests in series is needed to confirm the diagnosis because the high sensitivity of ELISA can result in some false positive results.

Sera were inactivated at 58 °C for 30 min before being used in the virus neutralisation (VN) and complement fixation (CF) tests. VN was performed for EAV, as prescribed for international trade by OIE (2). Briefly, VN was carried out using sterile 96-well flat-bottom, polyvinyl chloride microtitre plates. The sera were diluted 1:4 in a minimum essential medium with 10% foetal bovine serum, and serial 2-fold dilutions were incubated for 1 h at 37 °C with an equal volume of 100–300 $TCID_{50}$ of a *Bucyrus* reference strain of EAV. Approximately 1.5×10^4 RK-13 were put in the wells. The plates were incubated for 48–72 h at 37 °C in a humidified atmosphere with 5% CO_2 in the air. Equine sera controls for cell viability, serum cytotoxicity, viral infectivity, positive antibody, and negative antibody were included in each assay. The end-point VN titre was expressed as the reciprocal of the highest dilution of serum showing no cytopathic effects in any of the wells.

The CF test was performed to detect antibodies against *Burkholderia mallei* and *Trypanosoma equiperdum*, as prescribed for international trade by OIE (4,5). Each serum was initially screened at a dilution of 1:5 and checked for an eventual anticomplementary reaction. The samples with 100% haemolysis at the 1:5 dilution were considered negative, those with 25%–75% haemolysis were inconclusive, and those with no haemolysis, corresponding to a complete fixation, were positive.

2.3. Literature search

A literature search was carried out using Medline (from 1966), Science Citation Index Expanded (from 1946), and CAB abstracts (from 1975) up through December 2010. The search algorithm used was "(equine viral arteritis OR EAV OR equine arteritis virus); (equine infectious anaemia OR EIAV OR equine infectious anaemia virus); (dourine OR trypanosoma equiperdum); (glanders OR *Pseudomonas mallei* OR *Burkholderia mallei*); AND (Turkey OR Turkish)" across all fields. In addition, the World Animal Health Information Database (WAHID), the international database of the OIE, was checked to verify the official notification status of these diseases.

The bibliographies of all identified publications were checked to identify further relevant studies. No language restrictions were used in the searches. Abstracts were translated for publications in languages other than English. Acceptable studies were serological or diagnostic studies done on Turkish equid populations (horse, donkey, or others) in order to determine the presence of the diseases in the country.

3. Results

3.1. Serological tests

Out of the 346 tests performed for EIA, 317 sera had negative results, 22 were positive and 7 were inconclusive according to the ELISA screening test. The positive and inconclusive sera were all negative in the subsequent AGID test. In the test for antibodies against EAV, 57 sera out of 346 (16%) had positive results (Table). Forty-nine sera showed anticomplementary reactions and were not assessable with the CF test, whereas 297 had a negative result for glanders and dourine. The sera with anticomplementary activity were not submitted to the other tests because of the limited quantity of the samples.

Table. Results of the virus neutralisation (VN) test for equine viral arteritis from the 346 equine serum samples.

VN titre	No. of positive sera
1:4	14
1:8	14
1:16	9
1:32	13
1:64	3
1:128	4
Total	57

3.2. Literature search

For EIA, 6 international scientific references were found in electronic databases (6–11) and 2 by manual search (12,13). All these reports are serological investigations from various regions in Turkey and all sera tested (more than 11,000 equids) were negative for antibodies against EIAV. However, WAHID displayed 3 outbreaks during 2005 in Siirt and Şırnak provinces that involved 22 equids (http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation).

For EAV, 2 international scientific references were found in electronic databases (14,15) and 3 by manual search (16–18), and the estimated seroprevalence of the disease in these papers ranged from 8.75% (16) to 14% (14,15). WAHID did not provide information for the status of this disease (http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation).

For glanders, 3 international scientific references were found in electronic databases (19–21). Arun et al. (19) reported a positivity rate of 3.1% (35 of 1128 horses) using the mallein test. Another reference (20) was older and described the gross and histological findings in 2 horses with glanders at slaughter that had been imported to Italy from Turkey. WAHID indicated that the last notified case of glanders was in March 1998 (http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation). Starting in 2000, a glanders eradication project was undertaken and in 2001, glanders was eradicated in Turkey (21).

No references were found for dourine, and WAHID contained no references to it in Turkey (http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation).

4. Discussion

In this study, horses were positive only for EAV, with a 16% seroprevalence. This is in accordance with previous studies (14,15) that found similar seroprevalences in Turkey. It is important to know whether EAV is present in a country in order to manage the international movement of horses and the commercial use of their semen. The reporting of seroprevalence for EAV is globally inconsistent, ranging from 2% to 20% (14,22) depending on the country, its horse population, and the surveillance programmes undertaken in the country. The risk of EAV in Turkey is similar to that of other parts of the world and further studies on the 2 possible carriers of the infection, stallions, and semen, should be undertaken.

None of the samples were positive for EIA. This is in accordance with the previous research (6–13) that tested large equid populations. However, WAHID indicates that 3 outbreaks occurred in Turkey in 2005. It would be very useful to know the details of those outbreaks to understand

the possible origin and source of EIAV. Turkey does not seem to be at risk for EIA but the reason for this is not evident. The situation should be investigated to determine if vector or environmental causes are responsible for this. Further studies are needed to increase our knowledge of the ecology of infection in this country.

Glanders was eradicated in Turkey in 2001, and a serological investigation with sera sampled during 2004 confirmed this result. However, monitoring for glanders is important to maintain this eradication status, considering there are continuously new outbreaks throughout the world and in nearby countries such as Iran and Bahrain (http://www.aht.org.uk/cms-%20/disease_surveillance.html).

To the authors' knowledge, this is the first serological report of dourine in Turkey, and no positive serum was found. However, further epidemiological investigations should also be performed for dourine because outbreaks are still being reported around the world (http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation).

The present study is limited by the small population examined and that it was not randomly selected. Moreover, the young mean age could have biased the result, showing more negative results than actually exist because young animals have a lower probability of infection. Furthermore, all diagnostic tests have limitations in detecting infection; for example, these immunological tests can fail in the early stages of infection or in immunosuppressed animals.

The systematic review is a reproducible and rigorous method of searching all data published in the world about a topic in order to guarantee the completeness of the research for a defined topic. The systematic review of the diseases investigated in this study is useful to improve understanding of the overall situation in Turkey. However, further research is needed to trace the real status of every disease. For example, important data were not available for the outbreaks of EIA in 2005 because no investigations were reported. It might have been different if the outbreaks had occurred in imported or national horses. The systematic review, together with a serological investigation, can also be a useful tool to identify new points of research.

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