# Seroepidemiological survey of *Rhodococcus equi* infection in asymptomatic horses and donkeys from southeast Turkey

O Y Tel[a*], N B Arserim[b] and O Keskin[a]


## ABSTRACT

In order to assess the level of *Rhodococcus equi* infection in southeast Turkey, 679 sera from healthy foals and adult horses and 78 sera from donkeys were tested by indirect ELISA using a *R. equi* reference strain (ATCC 33701) as antigen. Eighty (11.7 %) sera from horses and 9 (11.5 %) sera from donkeys with titres >0.85 were positive. The prevalence of seropositive horses in Sanliurfa Province was higher than in Diyarbakir Province; 56 (13.9 %) horses in Sanliurfa Province and 24 (8.7 %) horses in Diyarbakir Province were defined as seropositive. In Sanliurfa Province 14.5 % of female ($n$ = 343) and 10.1 % of male ($n$ = 59) horses tested were defined as seropositive, while in Diyarbakir Province more males (11.4 %, $n$ = 114) were seropositive than females (6.7 %, $n$ = 163). Horses 1 to 5 years of age were found to have the highest seropositivity rate in both provinces. A total of 78 sera from donkeys were investigated in Sanliurfa Province, of which 9 (11.5 %) were positive by ELISA. Among the 9 positive sera, 6 (12.8 %) were from donkeys 1–5 years old and 3 (13.6 %) were from donkeys >5 years of age. No positive sera were found in donkeys less than 1 year old. Five(12.5 %) sera of females and 4(10.5 %) sera of males tested were positive. These results indicate the existence of *R. equi* in the horse populations in Sanliurfa and Diyarbakir Provinces. Similar infection rates were found for donkeys in Sanliurfa. This suggests the importance of serological surveys to diagnose *R. equi* infection in the region and to prevent the zoonotic risk.


**Keywords**: donkey, ELISA, foals, horse, *Rhodococcus equi.*

Tel O Y, Arserim N B, Keskin O **Seroepidemiological survey of *Rhodoccocus equi* infection in asymptomatic horses and donkeys from southeast Turkey**. *Journal of the South African Veterinary Association* (2011) 82(4): 224–226 (En.). Department of Microbiology, Faculty of Veterinary Medicine, Harran University, Sanliurfa, Turkey.

## INTRODUCTION

*Rhodococcus equi* infection was 1st described in foals in 1923[8]. It is currently recognised worldwide as a major cause of disease in foals of 3 weeks to 6 months of age[9]. Rhodococcal disease is uncommon in adult horses because most adults are immune to infection[13]. The most common clinical manifestation of *R. equi* infections in foals is pyogranulomatous pneumonia. Other reported clinical manifestations include ulcerative enterocolitis, colonic or mesenteric lymphadenopathy, immune-mediated synovitis and uveitis, osteomyelitis, and septic arthritis[2,4]. The organism is worldwide in distribution and commonly isolated from soil and environmental samples[11,15]. *R. equi* has been isolated from a wide variety of species, including cats, dogs, goats, cattle, camelids, pigs, crocodiles, and other animals[6]. *R. equi* is considered an important pathogen in immunocompromised human patients[11].

Early clinical diagnosis in foals is often difficult, therefore serological surveillance for *R. equi* infection in foals is necessary. The best way to limit the cost of therapy in stud farms with high contamination is to prevent the spread of virulent organisms. More recently, serological testing has been recommended primarily as a surveillance tool to identify foals suspected of being infected, particularly on farms on which the disease is endemic[4,5]. Serologic assays, developed to detect *R. equi*-specific antibodies, include several enzyme-linked immunosorbent assays (ELISAs), an agar-gel immunodiffusion (AGID) test, and synergistic haemolysis inhibition (SHI) assays[6]. Serological tests such as AGID, complement fixation and indirect haemagglutination assay (IHA) carried out on foals are considered to be of little or no value for diagnosing *R. equi* infection because they are not sensitive enough[3]. An ELISA test has recently been introduced for use in foals that is more sensitive than previously used methods for detecting antibodies against *R. equi*[3,5,14].

At present little information is available about the seroprevalence of *R. equi* in Turkey. Previously, 2 serological studies were carried out on Thoroughbred foals from the Marmara Region in Turkey[1,10]. The objective of this study was to determine seroprevalence of *R. equi* using horse and donkey sera from Sanliurfa and Diyarbakir Provinces, Southeast Turkey.

## MATERIALS AND METHODS

### *Animals*

A total of 679 healthy horses, (506 females and 173 males) and 78 healthy donkeys (40 females and 38 males), were selected to determine the presence of antibodies against *R. equi.* The serum samples were collected between the years 2009 and 2010. Horses (Thoroughbred, Arabian and half-bred) and donkeys ranged in age from 1 month to 20 years. In order to correlate the prevalence and the antibody titres with the age, the animals were subdivided into 3 age-groups: 1–12 months old, 1–5 years old and >5 years old. The presence of antibodies against *R. equi* was determined according to $OD_{450}$ positive ranges: negative titre ($OD_{450} \leq 0.85$) and positive titre ($OD_{450} > 0.85$).

### *Test procedure*

All sera were analysed using an ELISA test according to the method described by Takai *et al.*[14]. The ELISA antigen was prepared from *R. equi* ATCC 33701, a compound derived from the cell wall of *R. equi,* as described previously[14]. Briefly, bacteria were grown on brain-heart-infusion agar and harvested after 5 days of incubation at 38 °C. *R. equi* colonies (2 g, wet weight) were suspended in 10 mℓ of 0.0125 M sodium phosphate buffer (pH 7.4) containing 0.1 % (w/v) Tween 20. Solutions were incubated at 37 °C for 90 minutes in a water bath with agitation and centrifuged at 20 000 *g* for 30 min at 4 °C. The supernatant was used as an antigen, which was adjusted to 1.0 μg of protein/mℓ in carbonate-bicarbonate buffer (pH 9.6).

[a]Department of Microbiology, Faculty of Veterinary Medicine, Harran University, Sanliurfa, Turkey.

[b]Department of Microbiology, Faculty of Veterinary Medicine, Dicle University, Diyarbakir, Turkey.

*Author for correspondence. E-mail: oyasar@gmail.com

Received: July 2011. Accepted: October 2011.

Table 1: **Seroprevalence of healthy horses from different age-groups in Diyarbakir and Sanliurfa Provinces.**

| Age | Sanliurfa Province (*n* = 402) | | | Diyarbakir Province (*n* = 277) | | | Total positive |
|---|---|---|---|---|---|---|---|
| | Number of samples | Positive *n* ( %) | Negative *n* ( %) | Number of samples | Positive *n* ( %) | Negative *n* ( %) | |
| ≤1 | 146 | 17 (11.6 %) | 129 (89.5 %) | 63 | 5 (7.9 %) | 58 (92.1 %) | 22 |
| >1 to ≤5 | 81 | 13 (16.1 %) | 68 (83.9 %) | 110 | 13 (11.8 %) | 97 (88.2 %) | 26 |
| >5 | 175 | 26 (14.9 %) | 149 (85.1 %) | 104 | 6 (5.8 %) | 98 (94.2 %) | 32 |
| Total | 402 | 56 (13.9 %) | 346 (86.1 %) | 277 | 24 (8.7 %) | 253 (91.3 %) | 80 (11.7 %) |

Table 2: **Seroprevalence according to provenance and sex in horses.**

| Sex | Sanliurfa Province (*n* = 402) | | | Diyarbakir Province (*n* = 277) | | | Total positive |
|---|---|---|---|---|---|---|---|
| | Number of samples | Positive OD > 0.85 *n* ( %) | Negative OD ≤ 0.85 *n* ( %) | Number of samples *n* ( %) | ODP ≤ 0.85 *n* ( %) | OD > 0.85 | |
| Female | 343 | 50 (14.5 %) | 293 | 163 | 11 (6.7 %) | 152 (94.4 %) | 61 (12 %) |
| Male | 59 | 6 (10.1 %) | 53 | 114 | 13 (11.4 %) | 101 (88.5 %) | 19 (10.9 %) |
| Total | | | | | | | 80 (11.7 %) |

ELISA was carried out as previously described[14]. Between each of the 4 steps the Microtitre plates were washed using a phosphate-buffered saline solution (PBSS, pH 7.2) containing 0.02 % Tween 20 (200 $\mu\ell$/wash). Horse serum inactivated at 56 °C for 30 min and diluted in PBSS containing 10 % foetal calf serum was added to the wells. Plates were incubated at 37 °C for 60 min and washed. 100 $\mu\ell$ of anti-Horse IgG Peroxidase Conjugate (Sigma-Aldrich, Milan, Italy), diluted 1:80 000, was added to each well and the plates were incubated at 25 °C for 30 min. After addition of substrate solution (0.4 mg o-phenylenediamine dihydrochloride/ 1 m$\ell$ of phosphate–citrate buffer containing sodium perborate [pH 5.0]; Sigma-Aldrich,Milan, Italy), the plates were incubated at 25°C for 30 min. The reaction was stopped using 100 $\mu\ell$/well of 3N $H_2SO_4$. Optical density (OD) was measured using an ELISA reader (Molecular Device, VERSAmax) at 450 nm. Positive serum collected from an immunised horse ($OD_{450}$ 1.650) and negative serum ($OD_{450}$ 0.040) collected from a foal before suckling colostrum were used as controls. An OD450 value >0.85 was considered positive for the presence of antibodies against *R. equi*. The average was 0.580 and the standard deviation (SD) was 0.09.

The algorithm of OD was the mean of the negative control values plus 3 times the SD value, i.e. 0.85 was chosen as cut-off value[7].

## RESULTS

Exposure to *R. equi* infection as indicated by the presence of antibodies was diagnosed in 80 (11.7 %) of 679 horses, with 56 (13.9 %) horses in Sanliurfa Province and 24 (8.7 %) horses in Diyarbakir Province determined to be seropositive (Table 1). Seropositivity rates of 14.5 % and 10.1 % were determined for female ($n$ = 343) and male ($n$ = 59) horses from Sanliurfa Province, while in Diyarbakir Province the rate of infected males (11.4 %, $n$ = 114) was higher than in females (6.7 %, $n$ = 163) (Table 2). Horses aged 1–5 years were found to have the highest seropositivity rate in both provinces (Table 1).

In Sanliurfa Province 9 (11.5 %) of 78 sera from donkeys tested positive by ELISA (Table 3). Among the 9 positive sera, 6 (12.8 %) were from the 1–5 year age group and 3 (13.6 %) from donkeys older than 5 years. No positive sera were found in donkeys under 1 year of age. In female and male donkeys, 5 (12.5 %) and 4 (10.5 %) sera tested positive respectively (Table 4).

## DISCUSSION

*R. equi* is considered 1 of the most common causes of respiratory disease in foals younger than 6 months of age and is responsible for severe or chronic pyogranulomatous pneumonia[6]. Although the infection has a worldwide distribution, until now there have been few reports about the seroprevalence of *R. equi* infection in Turkey[1,10]. A serological assay would be of considerable benefit in regions with enzootic *R. equi* infection.

In this study, 679 sera from healthy foals and adult horses and 78 sera from donkeys were tested by indirect ELISA and

Table 3: **Seroprevalence of healthy donkeys from different age groups in Sanliurfa Province.**

| Age | Positive (OD ≤ 0.85) *n* ( %) | Negative (OD > 0.85) *n* ( %) | Total |
|---|---|---|---|
| ≤1 | 0 (0 %) | 9 (100 %) | 9 |
| >1 to ≤5 | 6 (12.8 %) | 41(87.2 %) | 47 |
| >5 | 3 (13.6 %) | 19 (86.4 %) | 22 |
| Total | 9 (11.5 %) | 69 (88.5 %) | 78 (100 %) |

Table 4: **Seroprevalence according to sex of healthy donkeys in Sanliurfa Province.**

| Sex | Positive (OD ≤ 0.85) *n* ( %) | Negative (OD > 0.85) *n* ( %) | Total |
|---|---|---|---|
| Female | 5 (12.5 %) | 35 (87.5 %) | 40 |
| Male | 4 (10.5 %) | 34 (89.5 %) | 38 |
| Total | 9 (11.5 %) | 69 (88.5 %) | 78 (100 %) |

80 (11.7 %) sera from horses and 9 (11.5 %) sera from donkeys were determined to be positive. A serological survey of *R. equi* infection in horses by an ELISA-test in Japan found that 11.0 % of horses were seropositive[12]. In another study, 696 sera were collected from healthy horses from Bursa, Izmir, and Istanbul Provinces of Turkey and 14.8 % were found to be seropositive[1]. In central Italy, serum samples from 602 foals aged 1–6 months were tested, revealing that 13.45 % of animals had antibodies against *R. equi*[3]. In a study in which 752 serum samples from foals showing different clinical signs of infectious disease were examined, a seroprevalence of 18.35 % was reported[5]. In Marmara Region of Turkey 46 % of 454 foals examined were seropositive[10].

The seroprevalence rates (11.7 % for horses and 11.5 % for donkeys) observed in this study indicate that natural exposure of healthy horses and donkeys to *R. equi* occurs in Sanliurfa and Diyarbakir Provinces. This result is in agreement with the earlier reported prevalence rates in horses[1,3,12]. However, the seroprevalence rates found in the present study were lower than those reported in some studies[5,10], probably owing to the use of sera from horses and donkeys without clinical signs in the present study.

In addition, more horses in Sanliurfa Province were seropositive than in Diyarbakir Province. The variation in the serological results, according to the different area, might be attributed to differences in environmental conditions such as temperature, dust, soil pH, and management factors[4,11,13].

In Sanliurfa Province, the number of seropositive male horses (10.1 %) was lower than that of females (14.5 %). Similar rates have been found in donkeys in Sanliurfa. However, Diyarbakir area had a higher seroprevalence in male horses. Other researchers[3,12] have reported that the seropositive rate of females was significantly higher than that of males, but a higher seropositivity rate in males than in females was found in Istanbul Province. The different prevalence rates according to sex observed in Diyarbakir and Sanliurfa Provinces could be a result of management practices and differences in environmental conditions.

The results showed that 11.7 % of the horses examined in this study were exposed to *R. equi,* leading to antibody production without showing clinical signs. Of these, 17 (11.6 %) were aged less than 1 year, 13 (16.1 %) were less than 5 years old, and 26 horses (14.9 %) were older than 5 years in Sanliurfa Province, while 5 (7.9 %), 13 (11.8 %) and 24 (8.7 %) were seropositive in the <1 year, 1–5 years, and >5 years age groups in Diyarbakir Province, respectively. Nevertheless, similar prevalences were recorded between the 1st and 3rd groups. The highest antibody titres were observed in the 2nd age group. These results are in agreement with those reported in Istanbul Province[1]. Failure to find seropositive donkeys under 1 year old could be attributed to the small sample size for these animals.

The results of this study showed that 11.7 % of the horses and 11.5 % of donkeys in southeastern Turkey had antibodies against *R. equi,* indicating that *R. equi* exists on horse farms in the region. Since excretions of animals infected with *R. equi* could become a source of infection for other foals as well as a hazard for human health, precautions to prevent *R. equi* infection should be taken on the horse farms of the region.

## ACKNOWLEDGEMENTS

This study was supported by Harran University Scientific Research Council (HUBAK, Project No: 1008). The authors would like to thank Prof. Dr Osman Erganis for his significant contribution to the research.

## REFERENCES

1. Attili A R, Kennerman E, Takai S, Or M E, Marenzoni M L, Torun S, Pieramati C, Kayar A, Golcu E, Parkan Ç, Yilmaz Z, Gonul R, Valente C, Cuteri V 2006 Seroepidemiological survey of *Rhodococcus equi,* infection in asymptomatic horses from Bursa, Izmir and Istanbul provinces, Turkey. *Comparative Immunology, Microbiology and Infectious Diseases* 29: 323–333
2. Chaffin M K, Cohen N D, Martens R J, Edwards R F, Nevill M, Smith R III. 2004 Hematologic and immunophenotypic factors associated with development of *Rhodococcus equi* pneumonia of foals at equine breeding farms with endemic infection. *Veterinary Immunology and Immunopathology* 100: 33–48
3. Cuteri V, Takai S, Moscati L, Battistacci L, Pieramati C, Valente C 2003 A serological survey of *Rhodococcus equi* infection in foals in central Italy: comparison of two antigens using an ELISA tests. *Comparative Immunology, Microbiology and Infectious Diseases* 26: 17–23
4. Giguere S, Prescott J F 1997 Clinical manifestations, diagnosis, treatment and prevention of *Rhodococcus equi* infections in foals. *Veterinary Microbiology* 56: 313–334
5. Higuchi T, Hashikura S, Gojo C, Inui T, Satoh S, Yoshida M, Ishiyama T, Yamada H, Takai S 1997 Clinical evaluation of the serodiagnostic value of enzyme-linked immunosorbent assay for *Rhodococcus equi* infection in foals. *Equine Veterinary Journal* 29: 274–278
6. Hines M. 2007 *Rhodococcus equi* In Sellon D C, Long M T (eds) *Equine infectious diseases.* Saunders, Elsevier, Missouri: 281–295
7. Lopez A M, Hines M T, Palmer G H, Knowles D P, Alperin D C, Hines S A 2003 Analysis of anamnestic immune responses in adult horses and priming in neonates induced by a DNA vaccine expressing the vapA gene of *Rhodococcus equi. Vaccine* 21: 3815–3825
8. Magnusson H 1923 Spezifische infektiose pneumoniae beim fohlen. Ein neuer Eitererreger beim Pferd. *Archiv für Wissenschaftliche Praktische Tierheilkunde* 50: 22–37
9. Meijer W G, Prescott J F 2004 *Rhodococcus equi. Veterinary Research* 35: 383–396
10. Ozgur N Y, Ikiz S, Bagcigil A F, Carioglu B, Akay O, Ilgaz A, Takai S 2002 Studies on early diagnosis of *Rhodococcus equi* infection by ELISA in foals from racehorse breeding stud farms in the Marmara Region. *Turkish Journal of Veterinary and Animal Sciences* 26: 1427–1434
11. Prescott J F 1991 *Rhodococcus equi:* an animal and human pathogen. *Clinical Microbiology Reviews* 4: 20–34
12. Sanada Y, Noda H, Nagahata H 1992 Serological survey of *Rhodococcus equi* infection in horses in Hokkaido. *Journal of Veterinary Medical Science* 54: 649–652
13. Takai S 1997 Epidemiology of *Rhodococcus equi* infections: a review. *Veterinary Microbiology* 56: 167–176
14. Takai S, Kawazu S, Tsubaki S 1985 Enzyme-linked immunosorbent assay for diagnosis of *Corynebacterium* (*Rhodococcus*) *equi* infection in foals. *American Journal of Veterinary Research* 46: 2166–2170
15. Weinstock D M, Brown A E 2002 *Rhodococcus equi*: an emerging pathogen. *Clinical Infectious Diseases* 34: 1379–1385