Seroprevalence of *Anaplasma phagocytophilum* in well-maintained horses from northern Tunisia

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†This paper is dedicated to the memory of our wonderful colleague Professor Abderrazek Ghorbel, who has enormously contributed in his field and passed away on October 1st, 2013.

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Abstract. The aim of the present study was to determine the seroprevalence of *Anaplasma phagocytophilum* in 343 well maintained horses belonging to 11 horse stables located in northern Tunisia with indirect immunofluorescence test. Overall, 16.3% (56/343) of tested animals were positive. Anti- *A. phagocytophilum* antibodies were present in horses located in all studied governorates except the governorate of Ben Arous. Nine horse stables out of 11 contained positive animals, the seroprevalence of each one varied from nought to 50%. Seroprevalence varied according to gender, breed and type of activity, contrary to age. Seroprevalence was higher in females than males (21.4 and 9.5% respectively). Similarly, seropositive animals belonging to the English thoroughbred breed was significantly higher (30.2%) than other breeds. According to the utilization of horses, the highest positivity rate (21.1%) was observed in brood mares. Our results demonstrate that *A. phagocytophilum* infection is widespread in well maintained horses from northern Tunisia, so equine granulocytic anaplasmosis should be suspected by veterinarians in front of suggestive clinical signs like fever, ataxia and reluctance to move.

INTRODUCTION


In horses, *A. phagocytophilum* infection is widespread in well maintained horses from northern Tunisia, so equine granulocytic anaplasmosis should be suspected by veterinarians in front of suggestive clinical signs like fever, ataxia and reluctance to move.
clinical signs of *A. phagocytophilum* infection in horses include high fever, lethargy, inappetence, staggering or ataxia, distal limb oedema, petechia, icterus and haematological alterations, such as thrombocytopenia, neutropenia, lymphopenia and mild anaemia (Franzén et al., 2009; Jahn et al., 2010; Giudice et al., 2011). Acute infections are self-limiting concurrently with the development of adaptive immunity and activation of macrophages (Rejmanek et al., 2012). Granulocytic anaplasmosis is characterized by dysfunction or suppression of host defenses, which predispose horses to develop opportunistic infections and secondary infections with bacteria, virus and fungi (Pusterla & Madigan, 2013).

Recently, the presence of *A. phagocytophilum* in ticks and various hosts has been reported throughout Africa, especially in Tunisia where infection by *A. phagocytophilum* has been reported in horses and dogs (Sarih et al., 2005; M’ghirbi et al., 2009, 2012). A study on the prevalence of equine granulocytic anaplasmosis in Tunisia was realized in outdoor grazing horses (M’ghirbi et al., 2012) but to our knowledge, no epidemiological study concerned well maintained horses.

Indirect immunofluorescent antibody assay (IFA) is the most commonly used test to confirm *A. phagocytophilum* infection (Bakken & Dumler, 2006). Antibodies appear 12-16 days after experimental infection of horses and persist for several months. A four-fold increase between paired serum samples is considered significant of an active infection (Franzén et al., 2005). The detection of increasing IgG titers during the acute and convalescent phase of infection may be helpful in establishing a diagnosis in the absence of molecular analysis (Passamonti et al., 2010).

The aim of the present study was to estimate the seroprevalence of *A. phagocytophilum* infection in well maintained horses in Northern Tunisia, and to evaluate the role of different risk factors.

**MATERIAL AND METHODS**

**Horses and study regions**
The study was performed from July to October 2009 on 343 horses belonging to 11 well maintained horse stables located in two regions: Great Tunis area and Bizerte. Great Tunis area consists of four governorates: Ariana, Manouba, Tunis and Ben Arous, and belongs to semi-arid bioclimatic zone with a mean annual rainfall of 400 mm. Bizerte area (governorate of Bizerte) belongs to sub-humid bioclimatic zone with a mean annual rainfall varying between 400 and 800 mm (Figure 1, Table 1). Horses belonged mainly to four breeds: Arabian (182), English Thoroughbred (53), Arabian-barb (53) and Barb (45) (Table 2). Other breeds were represented by small numbers: Ponies, Anglo-Arabian-barb, Breton-Barb, Mogod Pony and Belgian saddle horse. These horses were used for different activities: breeding, races, leisure and work (Table 2). The mean age of animals was 10.3 years (range: 1 to 32 years) and the animals were divided into five age groups according to their age (Table 1).

**Blood samples and indirect immunofluorescence test**
Horses were aseptically bled from jugular vein into dry tubes. The blood samples were centrifuged at 3,000 rpm for 10 min and sera were stored at -20°C until used. The presence of anti-*A. phagocytophilum* IgG antibodies was detected by a commercial *A. phagocytophilum* indirect immunofluorescence test kit (Fuller Laboratories, Fullerton, CA, USA). All samples were screened at the threshold dilution of 1:80 and positive samples were further titrated up to 1:320.

**Statistical analyses**
The chi-square or student t-tests were performed with Epi Info 6 to study the influence of horse stable, age, breed, gender and type of activity on *A. phagocytophilum* seroprevalence. The level of significance was set to 0.05.
Table 1. Horse’s distribution and seroprevalence of *Anaplasma phagocytophilum* among regions, governorates and stables

<table>
<thead>
<tr>
<th>Region</th>
<th>Governorate</th>
<th>Stable (ID number)</th>
<th>Seropositive/total (% ± 1.96 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Tunis</td>
<td></td>
<td></td>
<td>50/287 (17.4±0.04)</td>
</tr>
<tr>
<td>Ariana</td>
<td>1</td>
<td>2/20 (10.0±0.13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7/49 (14.3±0.10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5/31 (16.1±0.13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>14/100 (14.0±0.07)</td>
<td></td>
</tr>
<tr>
<td>Manouba</td>
<td>4</td>
<td>27/54 (50.0±0.13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0/27 (0.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3/41 (7.3±0.08)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30/122 (24.5±0.08)</td>
<td></td>
</tr>
<tr>
<td>Tunis</td>
<td>7</td>
<td>2/29 (6.9±0.09)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4/16 (25.0±0.21)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6/45 (13.3±0.10)</td>
<td></td>
</tr>
<tr>
<td>Ben Arous</td>
<td>9</td>
<td>0/20 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Bizerte</td>
<td></td>
<td></td>
<td>6/56 (10.7±0.08)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5/36 (13.9±0.11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>1/20 (5.0±0.10)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>56/343 (16.3±0.04)</td>
</tr>
</tbody>
</table>

1: Seroprevalence rate  
2: Standard deviation

Figure 1. Map of Tunisian studied breeding stables. (A): Map of Africa showing the location of Tunisia, (B): Map of Tunisia showing the location of Bizerte and Tunis regions and (C): Map of Bizerte region (stable numbers 10 and 11) and Great Tunis region showing the location of governorates of Ariana (stable numbers 1, 2 and 3), Manouba (stable numbers 4, 5 and 6), Tunis (stable numbers 7 and 8) and Ben Arous (stable number 9)
Table 2. Seropositive horses according to age, gender, breed and type of activity

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Group</th>
<th>Seropositive/total (%1 ± 1.96 SD2)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1-5 years</td>
<td>7/85 (8.2±0.06)</td>
<td>0.090</td>
</tr>
<tr>
<td></td>
<td>6-10 years</td>
<td>22/108 (20.4±0.08)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11-15 years</td>
<td>15/79 (19.0±0.07)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16-20 years</td>
<td>10/46 (21.7±0.12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21 years and more</td>
<td>2/25 (8.0±0.11)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>42/196 (21.4±5.7)</td>
<td>0.003*</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>14/147 (9.5±4.7)</td>
<td></td>
</tr>
<tr>
<td>Breed</td>
<td>Arabian thoroughbred</td>
<td>33/182 (18.1±0.05)</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>English thoroughbred</td>
<td>16/53 (30.2±0.12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barb</td>
<td>2/45 (4.4±0.06)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arabian-Barb</td>
<td>2/53 (3.8±0.05)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other breeds</td>
<td>3/10 (30.0±0.28)</td>
<td></td>
</tr>
<tr>
<td>Type of activity</td>
<td>Brood mares</td>
<td>46/217 (21.1±0.05)</td>
<td>0.015*</td>
</tr>
<tr>
<td></td>
<td>Leisure</td>
<td>6/32 (18.8±0.14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Race</td>
<td>1/15 (6.7±0.13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Equestrian brigade</td>
<td>2/49 (4.1±0.05)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Breeding (foals)</td>
<td>1/29 (3.4±0.07)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Work</td>
<td>0/1 (0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>56/343 (16.3±0.04)</td>
<td></td>
</tr>
</tbody>
</table>

1: Seroprevalence rate  
2: Standard deviation  
*: Significant test

RESULTS

A total number of 343 sera were analyzed by IFA, the overall seroprevalence was 16.3%, with 39 positive horses (11.4%) at the 1:80 dilution, 17 (5.0%) at the 1:160 dilution and no sera positive at the 1:320 dilution.

The seroprevalence in Great Tunis (17.4%) was higher than Bizerte (10.7%) but there was no statistically significant difference between areas (P = 0.214). The differences between seroprevalence observed in the different studied governorates were statistically significant (P = 0.017). The highest infection rate was observed in the governorate of Manouba (24.5%) followed by those of Ariana (14%) and Tunis (13.3%), while it was nil in Ben Arous (Table 1).

A horse stable was considered positive if at least one horse was seropositive. The seroprevalence in the different horse stables varied significantly from nought to 50% (Table 1) (P < 0.0001). There was no statistically significant difference between age categories (P = 0.090). Seropositivity rate was higher in females (21.4%) than males (9.5%) (P = 0.003).

The highest seroprevalence was observed in English thoroughbred (30.2%) followed by Arabian thoroughbred (18.1%) (P < 0.001) (Table 2). According to type of utilization of animals, the seroprevalence varied between nought and 21.1% for working horses and brood mares, respectively (Table 2) (P = 0.015).

DISCUSSION

In Tunisia, equine staff reaches 25,000 animals, with 20,000 Barb, 4,000 purebred Arabian and 1,000 Thoroughbred, as estimated by the National foundation of improvement of horse’s breeds (FNARC, Fondation nationale d’amélioration de la race
chevaline). In the present study, the seroprevalence rate in 343 well maintained horses from two areas of Tunisia, Great Tunis and Bizerte, was 16.3%. Recently, a study performed in Tunisia on horses grazing outdoor on permanent pastures and bushes showed a higher seroprevalence estimated at 67% (M’ghirbi et al., 2012). One important point explaining this difference could be the fact that latter horses were extensively reared; used for several forest duties, they graze in regions favourable for ticks. In addition, these animals are not treated with acaricides and were highly infested by ticks. On the contrary, during the present study, we did not find ticks on horses except one *Hyalomma scupense* male tick on a horse.

In addition, one of the main known vectors of *A. phagocytophilum* is *Ixodes ricinus* which has never been recorded in the studied areas. Therefore, it appears that other ticks or some biting insects may be involved in the transmission of the bacterium. Several studies reported that *A. phagocytophilum* seroprevalence rate differed by regions, related to suitable tick habitats and horse life style, promoting tick exposure in a particular locality (Egenvall et al., 2001; Leblond et al., 2005; Praskova et al., 2011; Giudice et al., 2012). In Sweden, quite similar seroprevalence of *A. phagocytophilum* in equids (17.0%) has been reported by Engvall & Engvall (2002). In Italy, the seroprevalence of *A. phagocytophilum* in horses ranged in different regions between 13 and 17% (Ebani et al., 2008; Passamonti et al., 2010; Laus et al., 2013), and in Denmark, it reached 22% (Hansen et al., 2010). However, in the Netherlands higher seroprevalence was reported (83.3%) (Butler et al., 2008). Moreover, in other European countries, low seroprevalences were observed such as in France (11.3%) (Leblond et al., 2005), Italy (0.3%; 7.8%) (Lillini et al., 2006; Torina et al., 2007), Spain (6.5%) (Amusategui et al., 2006) and Portugal (3%) (Santos et al., 2008).

Anti-*A. phagocytophilum* antibodies were detected in all studied governorates except Ben Arous and 9 out 11 horse stables contained positive animals (Table 1). The rate of positive stables (81.8%) is higher than in France (32.9%) (Leblond et al., 2005). Only two horse stables (n°5 and n°9) proved to be EGA-free. Horse stable n°5 is the only one which performed control of external acarids in animals and premises. In contrast, stable n°4 showed the highest rate of seropositivity (50%) (Table 1). It has no established protocol for tick control and, especially in hot season, horses spend the whole evening and night out the paddock. They are thus more likely exposed to bites of various blood sucking insects and ticks. Hansen et al. (2010) studied a possible correlation between the seroprevalence of *A. phagocytophilum* and the number of hours spent by horses outside boxes.

The present study showed no correlation between age and seroprevalence (Table 2) as reported by Leblond et al. (2005). In contrast, M’ghirbi et al. (2012) showed that the highest seroprevalence was observed in horses aged between 1 and 5 years. Hansen et al. (2010) reported that the seropositivity was higher in the two oldest horse groups (11 to 20 years and more than 21 years). The seroprevalence was significantly higher in females (21.4%) than males (9.5%). These observations are consistent with those of several studies (Egenvall et al., 2001; Leblond et al., 2005; Praskova et al., 2011; M’ghirbi et al., 2012). Egenvall et al. (2001) explained this higher seroprevalence reported in mares by the fact that the genders could be exposed differently to grazing and ticks, since older stallions are more troublesome when pastured than mares. However, these results disagreed with a study on Danish horses which reports no influence of gender on the seroprevalence of *A. phagocytophilum* (Leblond et al., 2005).

For results about the animal breeds, the present study showed that Thoroughbred horses (30.2%) were more likely to be seropositive than Arabian horses (18.1%). This difference between breeds was also observed by M’ghirbi et al. 2012 who reported higher seroprevalence in Barb horses than in Arabian horses. Leblond et al. (2005) found no significant difference between seroprevalence rates according to breed. In agreement with M’ghirbi et al. (2012) and unlike results of Leblond et al. (2005) and
Hansen et al. (2010), the present study shows that A. phagocytophilum infection risk is closely related to the horse’s activity, the highest rates being observed in brood mares and horses used in riding clubs.

This investigation confirmed that equine granulocytic anaplasmosis is present in Tunisia and physicians should be informed about that, so they can suspect the disease. In particular, the equine granulocytic anaplasmosis must be suspected in front of fever associated with depression, ataxia, reluctance to move, distal limb oedema and sometimes icterus and petechiation. The symptoms of granulocytic anaplasmosis are nonspecific and this could explain why the diagnosis is rarely made (Passamonti et al., 2010). Clinical anaplasmosis in horses is probably underdiagnosed as most horses recover spontaneously and clinical signs are similar to those caused by infections with other pathogens such as Borrelia burgdorferi, Babesia caballi, Theileria equi, equine herpesvirus, equine infectious anaemia virus, equine arteritis virus and Leptospiraceae (Butler et al., 2008).

All the positive horses were healthy and this is similar to a large serologic survey of 2,018 horses realized in Sweden that also included thorough veterinary clinical information, there were no statistical associations of positive A. phagocytophilum serology with clinical signs of disease (Egenvall et al., 2001).

Another explanation for the fact that the seropositive horses were healthy is that the blood samples were collected between July and August, while it seems that most infections occur in fall, winter, and spring, generally coinciding with peak adult tick (Madigan & Gribble, 1987; Ebani et al., 2008).

The prevalence of antibodies in the 343 healthy horses studied corroborates the observations of Passamonti et al. (2010), whereby the moderate/high rates of A. phagocytophilum antibody prevalence in healthy horses could possibly indicate that horses developed subclinical infection, and moreover subclinical infection or infection accompanied by mild clinical signs may be the most frequent outcome of infection in horses. This could explain why horses, but also dogs and humans, have been proposed as accidental hosts (Silaghi et al., 2011). The manifestation of the disease in animals vary according to the age, the variant of A. phagocytophilum involved, the host species and immunological status of the host (Stuen et al., 2013).

It would be very interesting to detect DNA of A. phagocytophilum by PCR and to identify the variants circulating in Tunisia, to study their diversity and virulence. Moreover, in Europe the isolates of human are clustered with those of horses and dogs, so the control of equine granulocytic in our country could have effects on human health.

The present study demonstrates that antibodies against A. phagocytophilum can be commonly found in Tunisian well maintained horses. Veterinarians should consider this pathological entity in the diagnostic and further studies are needed to assess the clinical prevalence of this disease. In addition, it would be very interesting to study the symptoms in diseased horses, to assess the serological and molecular prevalence of the A. phagocytophilum in other Tunisian regions and to investigate the vector competence of tick species or other parasites infesting horses.

Competing interests. The authors declare that they have no competing interests.

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