Seroprevalence of Paratuberculosis among Camels in Al-Ahsa and Riyadh Regions, Kingdom of Saudi Arabia

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ABSTRACT

Mycobacterium avium subsp. paratuberculosis infection causes reduction in milk yield, premature culling and reduced slaughter value. In this paper a seroprevalence of Paratuberculosis among camel in Al-Ahsa and Riyadh regions of the KSA was done. For this purpose a total of 444 camel sera samples were collected from Riyadh (n=101), and in the Eastern region from a Veterinary Clinic (n=171) and from Al-Hasa slaughter house (n=172). The sera were tested by ELISA for the detection of antibodies to Mycobacterium avium sub spp. paratuberculosis. Positive sera were 32 (7.2%), with 10 (9.9%) positive sera in the Riyadh region, while 22 sera (6.41%) were tested positive in the Eastern region. Statistical analysis showed no significant difference in the prevalence in the two regions. Prevalence of antibodies to Mycobacterium avium sub spp. paratuberculosis were statistically non-significant association of the camel breeds. Twelve (13.33%) seropositive camels were less than two years old and 20 (5.64%) were older than two years. The difference in prevalence in the two tested age strata was statistically significant. Five (5.1%) male and 27 females (7.8%) had antibodies to MAP. The difference between genders was not statistically significant. It was concluded that infection of camels with MAP occur in the KSA but not yet known to which extent they cause clinical disease. Further studies are needed to determine the risk factors of infection that influences a further spread of the infection. The determination of these risk factors will help to plan program to control the disease.


INTRODUCTION

Dromedary camel (Camelus dromedarius) is one of the highly valuable domestic animals in Saudi Arabia. Camels are multipurpose animals that can be used for meat, milk and wool production (Ali et al., 2009). The development of camel dairy farms and the booming of camel racing in the Guelph countries made camel as highly attractive commodity (Alhebabi and Alluwaim, 2010). Paratuberculosis is a chronic infection, which has been of particular concern in ruminants. The infection is caused by Mycobacterium avium subsp. paratuberculosis. The major effects of the infection on the animal level can be reduced milk yield (Kudahl et al., 2004), premature culling and reduced slaughter value (Benedictus et al., 1987), and losses due to continued spread of the infection (Kudahl et al., 2007; Salem et al., 2013). In Saudi Arabia, Paratuberculosis has been reported (Gameel et al., 1994; Al Hajri and Alluwaime, 2007). AL-Dubaib and Mahmoud, (2008) confirmed eighteen cases out of 90 goat herds (20%) (n=2610). Al-Naeem (2008) recorded an overall prevalence of 11.6% of paratuberculosis in sheep and goats (13.34% in goats and 10.38% in sheep) on the basis of serological test. Paratuberculosis is characterized by persistent and progressive diarrhea, weight loss, debilitation, and eventual death. Gross pathological examination of these cases revealed thickening of the intestinal wall up to three or four times the normal thickness, with corrugation of the mucosa which extended in some cases to the rectum. Mesenteric lymph nodes were moderately large and edematous (Tharwat et al., 2012). The diagnosis was confirmed by staining rectal smears by Ziehl-Neelsen stains. Alhebabi and Alluwaim (2010) confirmed the
spread of MAP infection in Saudi camel herds. They proved that the ruminant ELISA was useful for the screening of the MAP infection in camel and could rule out the need for the species specific ELISA test. Clinical and post-mortem lesions suspected the paratuberculosis in 5 camels in Quassim, Saudi Arabia. ELISA screening test used for detection of MAP in either animals or human (Dreier et al., 2006; Singh et al., 2008; Collins et al., 2011; Wadhwa et al., 2012; Fawzy et al., 2013) Ziehl-Neelsen staining of tissue scraping and tissue sections showed masses of acid fast bacilli, except for the spleen. Infection with Mycobacterium avium subsp. paratuberculosis was confirmed by PCR by targeting the IS900 or IS1245 genes (Thibault et al., 2006; Alharbi et al., 2011; Tuci et al., 2011; El Sayed 2014). Ghosh et al. (2012) identified isolates from diseased camels with Johne’s disease from the KSA using gene-based typing and found that all isolates belonged to sheep lineage of paratuberculosis suggesting transmission from infected sheep herds. This genome-wide analysis recognizes these camel isolates as a sub-lineage of the sheep strain with a significant number of single nucleotide polymorphisms (SNPs) between sheep and camel isolates (1000 SNPs). The economic losses due to disease beside the unknown extend in the Kingdom Saudi Arabia are essential factors to know the prevalence of infection in order to apply a suitable control program. The present paper describes the seroprevalence of infection of camels with MAP in in Al-Ahsa and Riyadh, Saudi Arabia.

MATERIALS AND METHODS

Sampling: Serum samples (for serological testing) were collected from the eastern province and Riyadh region in the Kingdom of Saudi Arabia by random according to (Thrusfield et al., 2001). Sample size was calculated on the basis of the following factors: i) total number of population in each region, ii) expected prevalence of 5%, iii) confidence level of 95% and iv) desired absolute precision of 5%. Total number of camels in Riyadh and Eastern province of the Kingdom of Saudi Arabia was 99598 and 20747, respectively.

The calculated minimum sample size was 73 camels from Riyadh and 73 from the Eastern province of the Kingdom of Saudi Arabia. We collected more samples for safety reasons and to be able to stratify the samples according to gender and age. Finally, a total number of 444 camel sera were collected from Riyadh (n=101), from a Veterinary Clinic in the Eastern region (n=171) and from Al-Hasa Slaughter house (n=172).

Collection of blood samples: Blood samples were obtained from the jugular vein using sterile syringes and vacutainer tubes during January till March 2010. They were allowed to clot at ambient temperature and centrifuged at 4000g for 15 minutes. Sera were then separated and stored at -20℃ until used.

Detection of antibodies to Mycobacterium avium subspecies paratuberculosis in camel sera by ELISA: A commercial ELISA for detecting serum antibodies to MAP in ruminants was used (Pourquier ELISA Paratuberculosis antibody screening, Montpellier, France) was applied. The test is an indirect ELISA, performed in microtiter wells coated with M. paratuberculosis antigen. Samples were first incubated with M. phlei extract to bind nonspecific antibodies. The pre-incubated samples were then transferred to the antigen coated plates so that M. paratuberculosis-specific antibodies could bind to the solid phase. Unbound material was removed by washing. Bound antibodies were detected by anti-ruminant horseradish peroxidase (HRPO) conjugate and TMB substrate was added for 10 min. The reaction was terminated by addition of 10 µl stop solution and the absorbance measured at a single wave length of 450 nm in a spectrophotometer. The calculation of S/P ratios and the cut off for the samples were done according to the instructions by manufacturer. Evaluation and interpretation of the results were followed according to the software (XHick Assay Management system, version: 3.3, Build: 252) provided kindly by IDEXX laboratories Inc.

Statistical analysis: Statistical and epidemiological analysis of the data collected were carried out by using SAS computer program and the essential epidemiological parameters according to (Thrusfield et al., 2001).

RESULTS AND DISCUSSION

Prevalence of antibodies to MAP in camel sera collected from Riyadh and the Eastern region of the Kingdom of Saudi Arabia illustrated in Table 1. The total number of positive sera were 32 (7.2%), with 10 (9.9%) positive sera in Riyadh region, while 22 (6.41%) positive in the Eastern region. Statistical analysis showed no significant difference in the prevalence between the two regions. Alhebabi and Alluwaimi (2010) investigated 386 camel sera and recorded 29 positive samples (3.73%). They believed that the results revealed fundamental findings regarding the relation of ELISA results to the pattern of MAP shedding. The most intriguing findings were the possibility of using ELISA as a forecasting tool for the commencement of shedding. Hussain et al., 2015 concluded that serologic tests especially ELISA are even less sensitive particularly in early stage or subclinically infected camels.

We also analyzed a potential association between breed and seropositivity to MAP in camel sera collected from Riyadh region (Table 2). The prevalence was 10.34, 00.00, 50.00, 9.80 and 7.14 in Maghater, Sheal, Soofer, Hegen and Magahem camel breeds, respectively. Statistical analysis revealed no significant association between the camel breeds and seropositivity to MAP. Okura et al. (2010) found an association between breeds and antibodies to MAP in adult Danish non-dairy cattle sampled at slaughter. The dairy breeds Danish Jersey, Danish Holstein and Danish Red Cattle ranked highest (i.e., with highest prevalence) (TP medians: 13, 10, and 6, respectively). Dairy breeds had a significantly higher prevalence than the others, median TP (dairy) = 15.7% vs. median TP (non-dairy) = 0.8%. For the individual non-dairy breeds, the median estimates were generally higher than the dairy.
Table 1: Prevalence of antibodies to Mycobacterium avium subspecies paratuberculosis in camel sera collected from Riyadh and the Eastern region, KSA

<table>
<thead>
<tr>
<th>Region</th>
<th>Number tested</th>
<th>Number of +ve</th>
<th>% of +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riyadh Region</td>
<td>101</td>
<td>10</td>
<td>9.9</td>
</tr>
<tr>
<td>Eastern Region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vet. Med. Hospital</td>
<td>171</td>
<td>15</td>
<td>8.77</td>
</tr>
<tr>
<td>Al-Hassa Slaughter house</td>
<td>172</td>
<td>7</td>
<td>4.06</td>
</tr>
<tr>
<td>Total</td>
<td>343</td>
<td>22</td>
<td>6.41</td>
</tr>
</tbody>
</table>

Chi square value = 4.25; P=0.11 OR = odds ratio; 95% CI= 95% confidence interval; Odds ratio of seroprevalence of mycobacterium avium between two regions was not significantly different [OR=1.603; 95% CI=0.73-3.50].

Table 2: Breed and MAP seropositivity in camel sera collected from Riyadh region

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number tested</th>
<th>Number of +ve</th>
<th>% of +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maghater</td>
<td>29</td>
<td>3</td>
<td>10.34</td>
</tr>
<tr>
<td>Sheal</td>
<td>5</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Soofer</td>
<td>2</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Hegen</td>
<td>51</td>
<td>5</td>
<td>9.8</td>
</tr>
<tr>
<td>Maghem</td>
<td>14</td>
<td>1</td>
<td>7.14</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>10</td>
<td>9.9</td>
</tr>
</tbody>
</table>

Chi square value = 4.33; P=0.36.

Table 3: Age and MAP seropositivity in camel sera collected from Al-Hassa and Riyadh regions

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of +ve</th>
<th>Number of –ve</th>
<th>% of +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two years and less</td>
<td>12</td>
<td>78</td>
<td>13.33</td>
</tr>
<tr>
<td>More than 2 years</td>
<td>20</td>
<td>334</td>
<td>5.64</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>412</td>
<td>7.20</td>
</tr>
</tbody>
</table>

Chi square value = 5.23; P=0.022 OR= odds ratio; 95% CI=95% confidence interval; Seroprevalence of mycobacterium avium was more prevalent in camels with age more than 2 years old than withcamels ≤ 2 years old [OR=2.569; 95% CI=1.20-5.47].

Table 4: Sex and MAP seropositivity in camel sera collected from Al-Hassa and Riyadh regions

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of +ve</th>
<th>Number of –ve</th>
<th>% of +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>5</td>
<td>93</td>
<td>5.10</td>
</tr>
<tr>
<td>Female</td>
<td>27</td>
<td>319</td>
<td>7.80</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>412</td>
<td>7.20</td>
</tr>
</tbody>
</table>

Chi square value = 0.47; P=0.48 OR= odds ratio; 95% CI=95% confidence interval; Odds ratio of seroprevalence of mycobacterium avium was not significantly different between male and female camels [OR=0.635; 0.238-1.696].

The interpretation of the effect of camel breeds on the prevalence of Mycobacterium avium subspecies paratuberculosis in infection is a matter of interest and needs a deep investigation and more sample size to clear the role of the genetic makeup of camels in disease resistance.

We also analyzed the potential effect of age on the seropositivity to MAP in camel sera (Table 3). Twelve (13.33%) seropositive camels were less than two years old and 20 (5.64%) were older than two years. The difference in prevalence in the two tested age groups was statistically significant (Chi square value = 5.23; P value = 0.022).

Five (5.1%) male and 27 (7.8%) female camels tested positive for MAP antibodies. Boadella et al. (2011) recorded a slight sex-related difference in sensitivity toward males free-range Eurasian wild boar (Sus scrofa scrofa) using the enzyme-linked immunosorbent assay for detecting antibodies to MAP. Again, the analysis of risk factors such as age for MAP in camels needs more investigations.

Conclusion: Infection with MAP occur in camels in the Kingdom of Saudi Arabia. More detailed studies are needed to investigate the potential effects on the health status of camels. Risk factors for infection need to be identified as well as environmental factors that might influence the spread of the disease. The determination of risk factors will help to plan the control of the infection if necessary.

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REFERENCES


