RESEARCH ARTICLE

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

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A B S T R A C T

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 Alluwaimi, 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 Alluwaimi (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.,* 2007; 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 strains of *Mycobacterium avium* sub spp. *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 -20ºC 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 (XChick 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 (3.37%). 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

Region	Number tested	Number of +ve	% of +ve
Riyadh Region	101	10	9.9
Eastern region			
Vet. Med. Hospital	171	15	8.77
Al-Hasa Slaughter house	172	7	4.06
Total	343	22	6.41
Total	444	32	7.2

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

Breed	Number tested	Number of +ve	% of +ve
Maghater	29	3	10.34
Sheal	5	0	0.0
Soofer	2	1	50
Hegen	51	5	9.8
Magahem	14	1	7.14
Total	101	10	9.9

Chi square value =4.33; P=0.36.

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

Age	Number of + ve	Number of – ve	% of +ve
Two years and less	12	78	13.33
More than 2 years	20	334	5.64
Total	32	412	7.20

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 camels with ≤ 2 years old [OR=2.569; 95% CI=1.20-5.47].

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

Age	Number of + ve	Number of – ve	% of +ve
Male	5	93	5.10
Female	27	319	7.80
Total	32	412	7.20

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 subsp. paratuberculosis* 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). Alhebabi and Alluwaimi (2010), who also showed that the ruminant ELISA could be used to detect *MAP*-specific antibodies in camels, screened 861 serum samples and found 29 positive (3.37%) and 17 suspect samples (1.97%) . However, they found 14 positive sera in the age group of 1-4 year-old camels (14 out of 386 samples; 1.62%). In the age group of 5-9 year-old animals, they detected only 5 positive in 268 sera (0.58%), while 10 positive in 207 (1.16%) from the group of animals that were 10-15 years old. These results are in accordance with

our findings suggesting that camels seroconvert early in their life to *MAP* if they are exposed. This is in contrast to situation in cattle which normally seroconvert only many months or even years after infection. As a consequence, the age-distribution among study objects is of great significance in the evaluation of a *MAP* antibody test for cattle. As an example, the probability of detecting infected cows that are two years of age using an antibody ELISA has been estimated to 0.06, whereas the same probability was 0.50 for cows of 5 years of age (Nielsen and Toft, 2006a). The immune response of camels in different stages of *MAP* infection in camels and the distribution of reactors and non-reactors require more detailed studies and in depth investigation.

The sex of camels also showed a statistically non-significant association with antibodies to *MAP* (Table 4). 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.

REFERENCES

Al-Dubaib MA and OM Moahmoud, 2008. Paratuberculosis of goats at Qassim region of central Saudi Arabia. Bulg J Vet Med, 11: 65-69.

Alharbi KB, A Al-Swailem, MA Al-Dubaib, E Al-Yamani, A Al-Naeem, M Shehata, ME Hashad, KA Albusadah and OM Mahmoud, 2012. Pathology and molecular diagnosis of paratuberculosis of camels. Trop Anim Health Prod, 34: 173-177.

Alhebabi AM and AM Alluwaimi, 2010. Paratuberculosis in camel (Camelus dromedarius): The diagnostic efficiency of ELISA and PCR. The Open Vet Sci J, 4: 41-44.

Al Hajri SM and AM Alluwaimi, 2007. ELISA and PCR for evaluation of subclinical paratuberculosis in the Saudi dairy herds. Vet Microbiol, 121: 384-385.

Ali I, MS Chaudhry and U Farooq, 2009. Camel rearing in Cholistan desert of Pakistan. Pak Vet J, 29: 85-92.

Al-Naeem A and Abdulmohsen, 2008. Seroprevalence of Paratuberculosis in Sheep and Goats in the Eastern Province of Saudi Arabia. J Egypt Vet Med Assoc, 68: 55-64.

Benedictus G, AA Dijkhuizen and J Stelwagen, 1987. Economic losses due to paratuberculosis in dairy cattle Vet Rec, 121: 142-146.

Boadella M, K Lyashchenko, R Greenwald, J Esfandiari, R Jaroso, T Carta, JM Garrido, J Vicente, J de la Fuente and C Gortázar, 2011. Serologic tests for detecting antibodies against Mycobacterium bovis and Mycobacterium avium subspecies paratuberculosis in Eurasian wild boar (Sus scrofa scrofa). J Vet Diagn Invest, 23: 77-83.

Collins MT, 2011. Diagnosis of paratuberculosis. Vet Clin North Am Food Anim Pract, 27: 581-591.

Dreier S, JI Khol, B Stein, K Fuchs, S Guetler and W Baumgartner. 2006. Serological, bacteriological and molecular biological survey of paratuberculosis (Johne's disease) in Austrian cattle. Vet Med, 53: 477-81.

El Sayed MSA, 2014. LCD array and IS900 efficiency in relation to traditional diagnostic techniques for diagnosis of *Mycobacterium avium subspecies paratuberculosis* in cattle in Egypt. Int J Mycobacteriol, 3: 101-107.

Fawzy A, A Prince, AA Hassan, A Fayed, M Zschöck, M Naga, M Omar, M Salem and A El-Sayed, 2013. Epidemiological studies on Johne's disease in ruminants and Crohn's disease in humans in Egypt. Int J Vet Sci Med, 1: 79-86.

Gameel AA, AS Ali, SA Razig, J Brown and A Elhendi, 1994. A clinico-pathological study on spontaneous paratuberculosis in camels (Camel Dromedaries). Pak Vet J, 14: 15-19.

Ghosh P, C Hsu, EJ Alyamani, MM Shehata, MA Al-Dubaib, A Al-Naeem, M Hashad, OM Mahmoud, KB Alharbi, K Al-Busadah, AM Al-Swailem and AM Talaat, 2012. Genome-Wide Analysis of the Emerging Infection with Mycobacterium avium Subspecies paratuberculosis in the Arabian Camels (*Camelus dromedarius*). PLoS ONE, 2: e31947.

Hussain MH, M Saqib , MG Al-Maawali, S Al-Makhladi, MS Al-Zadjali, T Al-Sidairi, S Asubaihi, A Al-Rawahi and MK Mansoor, 2015. Seroprevalence of Mycobacterium avium subspecies paratuberculosis (*MAP*) and evaluation of risk factors in camels of the Sultanate of Oman. Trop Anim Health Prod, 47: 383-389

Kudahl AB, SS Nielsen and JT Srensen, 2004. Relationship between antibodies against Mycobacterium avium subsp. Paratuberculosis. Prev Vet Med, 62: 119-134.

Kudahl AB, S Ostergaard, JT Sørensen and SS Nielsen, 2007. A stochastic model simulating paratuberculosis in a dairy herd. Prev Vet Med, 78: 97-117.

Nielsen SS and N Toft, 2006. Age specific characteristics of ELISA and fecal culture for purpose specific testing for paratuberculosis. J Dairy Sci, 89: 569-579.

Okura H, SS Nielsen and N Toft, 2010. Prevalence of Mycobacterium avium subsp. paratuberculosis infection in adult Danish non-dairy cattle sampled at slaughter. Prev Vet Med, 94: 185-190.

Salem M, C Heyde, A El-Sayed, SA Ahmed, M Zschöck and G Baljer, 2013. Mycobacterium avium subspecies paratuberculosis: an insidious problem for the ruminant industry. Trop Anim Health Prod, 45: 351-366.

Singh SV, AV Singh, R Singh, S Sharma, N Shukla, S Misra, PK Singh, JS Sohal, H Kumar, PK Patil, P Misra and KS Sandhu, 2008. Sero-prevalence of bovine Johne's disease in buffaloes and cattle population of North India using indigenous ELISA kit based on native Mycobacterium avium subspecies paratuberculosis 'Bison type' genotype of goat origin. Comp Microbiol Immunol Infect Dis, 31: 419-433.

Tharwat M, F Al-Sobayil, A Ali, M Hashad and S Buczinski, 2012. Clinical, ultrasonographic and pathologic findings in 70 camels (Camelus dromedarius) with Johne's disease. Can Vet J, 53: 543-8.

Thibault VC, M Grayon, ML Boschiroli, C Hubbans, P Overduin, K Stevenson, MC Gutierrez, P Supply and F Biet, 2007. New Variable-Number Tandem-Repeat Markers for Typing *Mycobacterium avium subsp. paratuberculosis* and *M. avium* Strains: Comparison with IS900 and IS1245 Restriction fragment length polymorphism typing. J Clin Microbiol, 45: 2404-2410.

Thrusfield M, C Ortega, I de Blas, JP Noordhuizen and K Frankena, 2001. Win Episcope 2.0: improved epidemiological software for veterinary medicine. Vet Rec, 148: 567-572.

Tuci A, F Tonon, L Castellani, A Sartini, G Roda, M Marocchi, A Caponi, A Munarini, G Rosati, G Ugolini, L Fuccio, M Scagliarini, F Bazzoli and A Belluzzi, 2011. Fecal detection of Mycobacterium avium paratuberculosis using the IS900 DNA sequence in Crohn's disease and ulcerative colitis patients and healthy subjects. Dig Dis Sci, 56: 2957-2962.

Wadhwa A, JP Bannantine, TM Byrem, TL Stein, AM Saxton, CA Speer and S Eda, 2012. Optimization of Serum EVELISA for Milk Testing of Johne's Disease. Foodborne Pathog Dis, 9: 749-754.