Journal of the Saudi Society of Agricultural Sciences xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of the Saudi Society of Agricultural Sciences

journal homepage: www.sciencedirect.com

Molecular detection of *Anaplasma marginale* in Arabian Camels (*Camelus dromedarius*) in Riyadh, Saudi Arabia

Emad Omer [a], Mohammed Elfehid [b], Abdelatiff Alwazan [b], Abdulaziz S. Alouffi [c], Fahdah A. Alshammari [d], Ibrahim Eldesoukey [e,f], Khaled Sultan [e,g,*]

[a] *CentralVeterinary Lab, Ministry of Environment, Water and Agriculture (MEWA), Riyadh, Saudi Arabia*
[b] *MEWA, 65 King Abdulaziz Road, Riyadh 11195, Saudi Arabia*
[c] *King Abdulaziz City for Science and Technology, Riyadh 12354, Saudi Arabia*
[d] *College of Science and Literature, Northern Borders University, Rafhaa 91911, Saudi Arabia*
[e] *Veterinary Diagnostic Lab-MEWA, Al-Baha Branch, King Abdulaziz Road, Al-Baha 65528, Saudi Arabia*
[f] *Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516 Kafr El-Sheikh, Egypt*
[g] *Department of Parasitology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516 Kafr El-Sheikh, Egypt*

ARTICLE INFO

Article history:
Received 15 February 2021
Revised 26 May 2021
Accepted 24 June 2021
Available online xxxx

Keywords:
Camel
Anaplasma marginale
PCR
MSP5
Saudi Arabia

ABSTRACT

Anaplasmosis, an underestimated tick-borne disease, is a common infection in ruminants including Arabian camels. To our knowledge, few studies have been carried out in Saudi Arabia to identify via molecular methods *Anaplasma* spp. that infect camels. Therefore, this study was performed to detect and identify *Anaplasma* spp. in Arabian camels in the Riyadh area of Saudi Arabia.

A total of 644 whole blood samples were collected between January 2018 and December 2019. Giemsa-stained, thin blood smears were examined microscopically and then a polymerase chain reaction (PCR) for selected positive samples had been carried out that targeted the specific region of major surface protein 5 (*MSP5*) of *Anaplasma marginale.*

A total of 273 out of 644 (42.39%) camels were found to be positive by microscopic examination for *Anaplasma* spp. Amplification of *MSP5* gene specific for *A. marginale* was done.

Results of the current study successfully detect *A. marginale* in camels in Saudi Arabia. To the best of our knowledge, this is the first molecular study that has confirmed the presence of *A. marginale* in Saudi camels. The *MSP5* gene provides a new attractive diagnostic target for detection of *A. marginale*. Also, the current study highlights the significant levels of camel anaplasmosis in Saudi Arabia.

1. Introduction

The one-humped or Arabian camel, *Camelus dromedarius,* is a multipurpose domestic animal that is distributed in specific parts of the world including the Middle East (Lbacha et al., 2017). In 2015, the camel population of Saudi Arabia was estimated at 1.4 million head, of which 23.2% were in the Riyadh area (Saudi General Authority for Statistics, 2015). In Saudi Arabia, camels are of strong cultural and socioeconomic significance, and the country is home to most of the camel population in the Arabian Gulf (Abdallah et al., 2012). Camel diseases receive significant attention because they are of veterinary interest and a public health concern (Abbas and Omer, 2005; Hemida et al., 2014).

Anaplasma species are tick-borne blood rickettsial pathogens that cause anaplasmosis, a wasting disease of livestock and particularly of ruminants such as cattle, camels, sheep and goats. Anaplasmosis cause considerable economic losses, especially in tropical and subtropical regions (OIE, 2015). In the past, *Anaplasma* species have been considered to be protozoan parasites, but recently after molecular analysis they have been classified as rickettsial bacterial pathogens in the Anaplasmataceae family (Dumler et al., 2001).

* Corresponding author at: Department of Parasitology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516 Kafr El-Sheikh, Egypt & Veterinary Diagnostic Lab-MEWA, Al-Baha Branch, King Abdulaziz Road, Al-Baha 65528, Saudi Arabia.
E-mail address: Khaled.soultan1@vet.kfs.edu.eg (K. Sultan).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jssas.2021.06.010
1658-077X/

Please cite this article as: E. Omer, M. Elfehid, A. Alwazan et al., Molecular detection of *Anaplasma marginale* in Arabian Camels (*Camelus dromedarius*) in Riyadh, Saudi Arabia, Journal of the Saudi Society of Agricultural Sciences, https://doi.org/10.1016/j.jssas.2021.06.010

Anaplasma species are intracellular blood pathogens that cause extravascular haemolysis. Genus *Anaplasma* includes, but is not limited to, the following species: *A. marginale*, *A. centrale*, *A. ovis*, *A. platys* and *A. phagocytophilum*. *A. marginale* is the causative agent of bovine anaplasmosis and infects dromedaries and cervids (Li et al., 2015). However, recent studies identified *A. marginale, A. platys, A. phagocytophilum* and *Candidatus Anaplasma camelii* from camels in Saudi Arabia and other parts of the world on the basis of parasitological, serological and molecular methods (Bastos et al., 2015; Li et al., 2015; Lbacha et al., 2017; Alanazi et al., 2020; Alshaharni et al., 2020).

Clinical diagnosis of animal anaplasmosis depends on signs that can overlap with those of other diseases. However, it is diagnosed in the laboratory mainly through examination of thin blood smears that are stained with Giemsa and/or Leishman stains and through use of serological tests such as competitive enzyme-linked immunosorbent assay (cELISA) to detect *Anaplasma* antibodies in sera (OIE, 2015). These diagnostic methods have disadvantages such as low sensitivity and lack of discrimination between different *Anaplasma* spp. Molecular methods that can be used to detect *Anaplasma* DNA by polymerase chain reaction (PCR) are more sensitive and specific to species level, and can be used for further phylogenetic analyses (Sudan et al., 2014; Parvizi et al., 2020). Another important point should be taken in consideration regarding diagnosis of animal anaplasmosis particularly *A. marginale*. Animals that pass the acute phase of the disease become "carrier" with persistent infection and usually do not develop clinical signs "asymptomatic" (Kocan et al., 2010). For diagnosis, using of Giemsa stained blood smears is not suitable in carrier animals, but serological and molecular based tools are more accurate (Noaman et al., 2009; Kocan et al., 2010, Bisen et al., 2021). In camels this situation is extended as anaplasmosis usually subclinical (Sudan et al., 2014).

Although camel anaplasmosis is of increasing research interest to identify the species and other factors of the disease, few studies have been conducted in Saudi Arabia (Ismael et al., 2016; Alanazi et al., 2020; Alshaharni et al., 2020).

The aims of this study were to estimate the detection rate of camel anaplasmosis in the Riyadh area and to identify the species involved in this disease via parasitological and molecular examination.

2. Materials and methods

2.1. Study area and samples

Riyadh Province is in the centre of Saudi Arabia (24°38′N46°43′E). It is the second largest district and is inhabited by about 25% of the country's population.

The study was carried out from January 2018 to December 2019. The samples were collected by Ministry of Environment, Water and Agriculture (MEWA) veterinarians and samples were tested in the MEWA central veterinary diagnostic laboratory. A total of 644 samples were collected (494 during 2018 and 150 during 2019). Approximately 5 ml of blood was collected directly from the jugular vein of dromedary camels that lived in different localities of the study area. The blood was drawn into clean tubes that contained ethylenediamine tetraacetic acid (EDTA) anticoagulant. Each sample was individually labeled and sent to the lab soon after collection.

2.2. Parasitological examination

Thin blood smears were prepared, stained with Giemsa and examined microscopically for the presence of intra-erythrocytic *Anaplasma*-inclusion bodies (Coetzee et al., 2005; Sharma et al., 2013).

2.3. DNA extraction

From selected samples that were found to be positive for *Anaplasma*-inclusion bodies by microscope examination, genomic DNA was extracted from the whole blood using the Magna Pure Compact kit (Roche®, Germany) according to the manufacturer's instructions. DNA was stored at −20 °C until amplification, for which it was used as a DNA template.

2.4. Molecular detection of MSP5 from Anaplasma marginale

All extracted DNA was amplified and the *MSP5* gene of *A. marginale* was targeted through use of the specific primer set (AMF): 5́-ACAGGCGAAGAAGCAGACAT-3́ and (AMR): 5́-ATAAATGGGAA CACGGTGGA-3́ (Ganguly et al., 2018). The PCR amplifications were performed in the Eppendorf Mastercycler® thermocycler (Eppendorf, Hamburg, Germany) in a total reaction volume of 25 μl that consisted of 1 μl of each primer, 2.5 μl of Fast Start DNA Master (HybProbe Version 16.Roche®, Germany), 1 μl $MgCl_2$, 5 μl of template DNA and 14.5 μl of DNA-free water. The amplification for the *MSP5* gene was performed according to the following steps: initial denaturation at 98 °C for five minutes, followed by 35 cycles of denaturation at 98 °C for two seconds each, annealing at 53 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension at 72 °C for one minute. The PCR products were loaded on to 2% agarose gel that was stained with 0.5 μg/ml ethidium bromide. The amplified DNAs were electrophoresed at 100 V for 60 min on a horizontal electrophoresis unit. The gel was then visualized and photographed using the UviTec Gel documentation system. To determine the fragment sizes, a100bp-plus DNA ladder was used.

2.5. Ethical considerations

All procedures were carried out in accordance with national laws and regulations for the handling of animals to avoid harm and minimize their pain. Veterinarians and the animals' owners were informed of the results.

3. Results

In the present study, microscopic examination of thin blood smears stained with Giemsa stain revealed *Anaplasma* spp. in 273 out of 644 samples (an overall detection rate of 42.39%). Among the samples taken in 2018, 210 of 494 samples were positive (42.51%) and among those that were taken in 2019, 63 out of 150 samples were positive (42%). *Anaplasma* spp. were observed within the infected erythrocytes and appeared as reddish-violet, pleomorphic, dot-like forms (0.2–0.4 μm diameter) (Fig. 1). For isolates that were used in the genetic study, a band of 382 bp was successfully produced that was specific for *MSP5* of *A. marginale* (Fig. 2).

4. Discussion

Anaplasmosis is a haemolytic, tick-borne disease that is common among ruminants in the tropics and subtropics, including the Middle East (Soosaraei et al., 2020). It is clinically characterised as per-acute to chronic and its infection of animals causes major economic losses (Sharma et al., 2013; Sudan et al., 2014). The most common species that infect domestic ruminants are *A. marginale*, *A. centrale*, *A. caudatum* and *A. ovis*. Of these, *A. marginale*is said to be the most pathogenic (Soulsby, 2005; Sudan et al., 2014). The taxo-

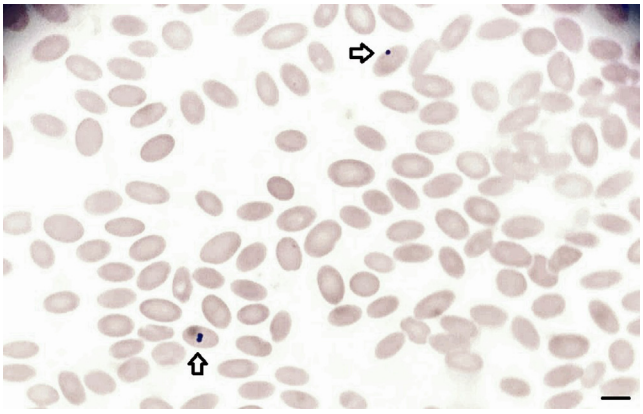

Fig. 1. Micrograph of Giemsa-stained thin blood smears of camels from Riyadh, Saudi Arabia. It shows *Anaplasma*-intraerythrocytic inclusion bodies as reddish-violet, pleomorphic, dot-like forms (0.2–0.4 μm diameter).

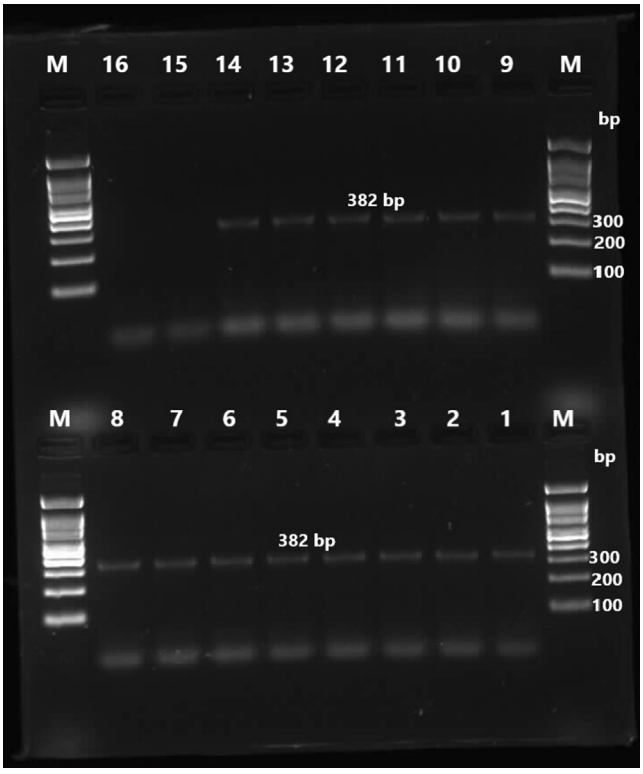

Fig. 2. Analysis of PCR product by electrophoresis on 2% agarose gel stained with ethidium bromide shows a successful amplification of specific *MSP5 Anaplasma marginale* gene. Lanes 1–14: *A. marginale* Saudi camel isolates, a positive band of 382 bp; lanes 15–16: negative control (no DNA); lane M: 100–1000 bp molecular weight marker.

nomic status of *Anaplasma* spp. that infect dromedaries remains uncertain and the subject is controversial.

The overall detection rate of *Anaplasma* that was found in the current study was higher than that reported (40.5%) by Ismael et al. (2016), who also examined blood smears taken from Arabian camels in Saudi Arabia. In their study,Ismael et al. (2016) identified the species as *A. marginale*. Result herein was also higher than that reported in Egypt (34.1%) by researchers who used cELISA (Parvizi et al., 2020), these researchers did not identify the species. Our result was higher than that recorded in Morocco (39.62%) by Lbacha et al. (2017), who used PCR and sequencing to target the *groEL* gene for Anaplasmataceae. They found the species *Candidatus A. camelii,* which is more closely related to *A. platys* than to *A. marginale*. In camels from the Asir area of Saudi Arabia, Alshahraniet al. (2020) used PCR and sequencing that targeted 16S and 23S rRNA of Anaplasmataceae, and found the species *Candidatus A. camelii* at a detection rate of 2%. More recently, Alanazi et al. (2020) found a 6.5% detection rate in camels in Riyadh Province. The species was *Anaplasma* spp. (i.e. *A. platys, A. phagocytophilum* and *Anaplasma* sp.). Li et al. (2015) reported *A. platys* from Bactrian camels *(Camelus bactrianus)* in China at a prevalence rate of 7.2%.

Several factors such as sample size, sampling procedures, examination method, abundance of vector ticks in the study area, and environmental and animal management conditions may be responsible for the variations in anaplasmosis prevalence or detection rates that have been reported in different studies. The blood samples that were studied in the current study were collected by veterinarians who suspected "blood parasite infection", and this purposeful sampling may explain the high rate of detection that is reported. However, according to the literature (Sudan et al., 2014; Ismael et al., 2016; Lbacha et al., 2017), *A. marginale* is the only species of *Anaplasma* that can produce a disease condition in dromedaries which is consistent with our findings.

At least four species of *Anaplasma* spp. (*A. marginale, A. platys, A. phagocytophilum,* and *Candidatus A. camelii*) have been confirmed to infect dromedaries. Yet camel infection with *A. marginale* has been primarily detected through conventional blood examination via stained blood smears, while other *Anaplasma* species in camels have been identified serologically or molecularly (Sudan et al., 2014; Li et al., 2015; Ismael et al., 2016; Lbacha et al., 2017; Alshahrani et al., 2020). Azmat et al. (2018) used amplification, sequencing and phylogenetic tree construction based on 16S rRNA of Anaplasmataceae to identify infection of Pakistani dromedaries with isolates that were genetically closer to *A. marginale* as well as isolates close to other species, including *A. phagocytophilum, A. platys, A. ovis,* and *Candidatus A. camelii*. Azmat et al. (2018) were the first researchers to use molecular methods to report and confirm *A. marginale* presence in dromedaries.

The *MSPs* are highly conserved genes within Anaplasmataceae. *A. marginale-MSP5* is a good target for specific identification and construction of informative phylogenetic trees (Corona et al., 2009; Machado et al., 2016;Ganguly et al., 2018). Recombinant *A. marginale-MSP5* has been used as an antigen in cELISA for the detection of specific *Anaplasma* antibodies in animal sera, although immune cross-reactivity has been observed between *A. marginale* and *A. phagocytophilum* (Knowles et al., 1996; de la Fuente et al., 2005). *A. marginale-MSP5* could be a target for vaccine production (Moladet al., 2004). Also, *A. marginale-MSP5* can be used to detect carrier animals (Bisen et al., 2021). To the best of our knowledge, successful amplification of *A. marginale-MSP5* in the current study offers the first molecular proof of *A. marginale* detection in Saudi dromedaries, and the results agree with those of Azmatet al. (2018) that camels can be infected by *A. marginale*. However, further sequencing of PCR products that were obtained in the present study is required to help to explain the status of anaplasmosis in Saudi Arabia and to classify specifically the species that are involved through use of other gene targets and samples from more ruminant species and of ticks.

In conclusion, *Anaplasma* spp. were detected in Arabian camels in the Riyadh area of Saudi Arabia. The identity of the species was confirmed to be *A. marginale* by amplification of the specific *MSP5 gene*. Further studies on the prevalence, molecular features and phylogenetics of *Anaplasma* spp. in Arabian camels and other animals in Saudi Arabia should be conducted, and effective control programmes should be carried out.

Funding

No special grant/fund was applied for this study. All devices, equipment, chemicals and kits were provided by MEWA, KSA.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to all veterinarians in the Riyadh branch of the MEWA as well as members of the MEWA Veterinary Diagnostic Laboratory, Riyadh, for their help and cooperation during this study. No special grant/fund was used in this study. All devices, equipment, chemicals and kits were provided by MEWA.

References

Abbas, B., Omer, O.H., 2005. Review of infectious diseases of the camel. Vet. Bulletin 75 (8), 1–16.

Abdallah, H.R., Faye, B., 2012. Phenotypic classification of Saudi Arabian camel (*Camelus dromedarius)* by their body measurements. Emir. J. Food Agric. 24 (3), 272–280.

Alanazi, A.D., Nguyen, V.L., Alyousif, M.S., Manoj, R.R., Alouffi, A.S., Donato, R., Sazmand, A., Mendoza-Roldan, J.A., Dantas-Torres, F., Otranto, D., 2020. Ticks and associated pathogens in camels (*Camelus dromedarius*) from Riyadh Province. Saudi Arabia. Parasit. Vectors 13 (1), 1–9.

Alshahrani, M.Y., Alanazi, A.D., Alouffi, A.S., Abdullah, H.H.A.M., Allam, A.M., Mahmoud, M.S., Abdel-Shafy, S., Alfaifi, M.H., Alkhathami, A.G., 2020. Molecular detection of *Candidatus Anaplasma camelii* in camels (*Camelus dromedarius*) from Asir Province. Saudi Arabia. Tropical Biomed. 37 (3), 587–598.

Azmat, M., Ijaz, M., Farooqi, S.H., A.,Ghaffar, A., Ali, A.,Masud, A.,Saleem, S.,Rehman, A., Ali,M.M., Mehmood, K., Khan, A., Zhang, H., 2018. Molecular epidemiology, associated risk factors, and phylogenetic analysis of anaplasmosis in camel. Microbial Pathogens.10.1016/j.micpath.2018.07.034.

Bastos, A.D., Mohammed, O.B., Bennett, N.C., Petevinos, C., Alagaili, A.N., 2015. Molecular detection of novel Anaplasmataceae closely related to *Anaplasma platys* and *Ehrlichiacanis* in the dromedary camel (*Camelus dromedarius*). Vet. Microbiol. 179 (3–4), 310–314.

Bisen, S., Aftab, A., Jeeva, K., Silamparasan, M., Yadav, S., Chandra, D., Sankar, M., Garg, R., Raina, O.K., 2021. Molecular and serological detection of *Anaplasma* infection in carrier cattle in north India. Vet. Parasitol. Reg. Stud. Reports 24, 100550.

Coetzee, J.F., Apleya, M.D., Kocanb, K.M., Rurangirwac, F.R., Van Donkersgoedd, J., 2005. Comparison of three oxytetracycline regimens for the treatment of persistent *Anaplasma marginale* infections in beef cattle. Vet. Parasitol. 127 (1), 61–73.

Corona, B., Machado, H., Rodríguez, M., Martínez, S., 2009. Characterization of recombinant MSP5 *Anaplasma marginale* Havana isolate. Brazil. J. Microbiol. 40 (4), 972–979.

de la Fuente, J., Lew, A., Lutz, H., Meli, M.L., Hofmann-Lehmann, R., Shkap, V., Molad, T., Mangold, A.J., Almazán, C., Naranjo, V., Gortázar, C., 2005. Genetic diversity of *Anaplasma* species major surface proteins and implications for anaplasmosis serodiagnosis and vaccine development. Ani. Health Res. Rev. 6 (1), 75–89.

Dumler, J.S., Barbet, A.F., Bekker, C.P., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and'HGEagent'as subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evol. 51 (6), 2145–2165.

Ganguly, A., Maharana, B.R., Ganguly, I., Kumar, A., Potlya, S., Arora, D., Bisla, R.S., 2018. Molecular diagnosis and haemato-biochemical changes in *Anaplasma marginale* infected dairy cattle. Indian J. Anim. Sci. 88 (9), 989–993.

Hemida, M.G., Chu, D.K., Poon, L.L., Perera, R.A., Alhammadi, M.A., Ng, H.Y., Siu, L.Y., Guan, Y., Alnaeem, A., Peiris, M., 2014. MERS coronavirus in dromedary camel herd. Saudi Arabia. Emerg. Infect. Dis. 20 (7), 1231.

Ismael, A.B., Swelum, A.A.A., Khalaf, A.F., Alowaimer, A.N., 2016. First evidence of natural anaplasmosis in *Camelus dromedarius* in Saudi Arabia. J. Camel Pract. Res. 23 (1), 95–100.

Knowles, D., De Echaide, S.T., Palmer, G., McGuire, T., Stiller, D., McElwain, T., 1996. Antibody against an *Anaplasma marginale* MSP5 epitope common to tick and erythrocyte stages identifies persistently infected cattle. J. Clin. Microbiol. 34 (9), 2225–2230.

Kocan, K.M., de la Fuente, J., Blouin, E.F., Coetzee, J.F., Ewing, S.A., 2010. The natural history of *Anaplasma marginale*. Vet. Parasitol. 167 (2–4), 95–107.

Lbacha, H.A., Zouagui, Z., Alali, S., Rhalem, A., Petit, E., Ducrotoy, M.J., Boulouis, H.J., Maillard, R., 2017. "*Candidatus anaplasma camelii*" in one-humped camels (*Camelus dromedarius*) in Morocco: a novel and emerging anaplasma species? Infect. Dis. Poverty 6 (1), 1–8.

Li, Y., Yang, J., Chen, Z., Qin, G., Li, Y., Li, Q., Liu, J., Liu, Z., Guan, G., Yin, H., Luo, J., 2015. *Anaplasma* infection of Bactrian camels (*Camelus bactrianus*) and ticks in Xinjiang. China. Parasit. Vectors 8 (1), 1–6.

Machado, R.Z., Teixeira, M.M.G., Rodrigues, A.C., André, M.R., Gonçalves, L.R., da Silva, J.B., Pereira, C.L., 2016. Molecular diagnosis and genetic diversity of tick-borne Anaplasmataceae agents infecting the African buffalo *Syncerus caffer* from Marromeu Reserve in Mozambique. Parasit. Vectors 9 (1), 1–9.

Molad, T., Brayton, K.A., Palmer, G.H., Michaeli, S., Shkap, V., 2004. Molecular conservation of MSP4 and MSP5 in *Anaplasma marginale* and *A. centrale* vaccine strain. Vet. Microbiol. 100 (1–2), 55–64.

Noaman, V., Shayan, P., Aminina, N., 2009. Molecular diagnostic of *Anaplasma marginale* in carrier cattle. Irani J. Parasitol. 4 (1), 26–33.

OIE, 2015.Terrestrial Manual.Section 2.4.Bovine, Chapter 2.4.1.Bovine Anaplasmosis. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=9&ved=2ahUKEwj_x4Di_Y7nAhU6QxUIHREpAfgQFjAIegQICBAB&url=https%3A%2F%2Fwww.baphiq.gov.tw%2Fuserfiles%2F2_4_1_%2520Bovine%2520anaplasmosis.pdf&usg=AOvVaw121fv5Mwg9tK-O98oE_wQ1

Parvizi, O., El-Adawy, H., Roesler, U., Neubauer, H., Mertens-Scholz, K., 2020. Performance Analysis of *Anaplasma* Antibody Competitive ELISA Using the ROC Curve for Screening of Anaplasmosis in Camel Populations in Egypt. Pathogens 9 (3), 165.

Saudi General Authority for Statistics. Detailed Results of the Agriculture Census, 2015.https://www.stats.gov.sa/en/1311.

Sharma, A., Singla, L.D., Kaur, P., Bal, M.S., Batth, B.K., Juyal, P.D., 2013. Prevalence and haemato-biochemical profile of *Anaplasma marginale* infection in dairy animals of Punjab (India). Asian Pac. J. Trop. Med. 6 (2), 139–144.

Soosaraei, M., Haghi, M.M., Etemadifar, F., Fakhar, M., Teshnizi, S.H., Asfaram, S., Esboei, B.R., 2020. Status of *Anaplasma* spp. infection in domestic ruminants from Iran: A systematic review with meta-analysis. Parasite Epidemiol. Control e00173.

Soulsby, E.J.L., 2005Helminths,. Helminths, arthropods and protozoa of domesticated animals. ELBS and Bailliere Tindal, London, pp. 752–754.

Sudan, V., Sharma, R.L., Borah, M.K., 2014. Subclinical anaplasmosis in camel (*Camelus dromedarius*) and its successful therapeutic management. J. Parasitic Dis. 38 (2), 163–165.