Aus dem Institut für Tier- und Umwelthygiene
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

in Kooperation mit
Friedrich-Loeffler-Institut - Institut für Bakterielle Infektionen
und Zoonosen, Jena

Overview of Anaplasmosis in Arab Countries in North Africa and the Middle East, and Optimizing a commercial c-ELISA for Camels

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der
Freien Universität Berlin

vorgelegt von
Omid Parvizi
Tierarzt aus Sanandaj/IRAN

Berlin 2020
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Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Jürgen Zentek
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Deskriptoren (nach CAB-Thesaurus):
anaplasma marginale, bovine anaplamosis, coxiella burnettii, prevalence, camelus, ELISA, real time pcr, egypt, north africa, middle east

Tag der Promotion: 07.10.2020

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Abbreviations

AOAD	: Arab Organization for Agricultural Development, Sudan
BA	: Bovine Anaplasmosis
CAPMAS	: Central Agency for Public Mobilization and Statistics, Egypt
CDC	: Centers for Disease Control and Prevention, USA
cELISA	: Competitive ELISA
IFA	: ImmunoFluorescent Assay
IUMS	: International Union of Microbiological Societies, Netherlands
NCBI	: National Center for Biotechnology Information, USA
n.a.	: Not applicable
n.d.	: not determined
OIE	: World Organization for Animal Health, France
ROC	: Receiver Operating Characteristic
UN	: United Nations, Switzerland
WHO	: World Health Organization, Switzerland
WBG	: World Bank Group, USA
WITS	: World Integrated Trade Solution (soft ware by WBG)

Introduction

The most common objective of veterinary public health research in the field of tick borne zoonoses is the promotion of animal welfare and reduction of economic losses by tailored countermeasures. *Anaplasma* species endanger the welfare and health of animals and can be potentially transmitted to humans. Anaplasmae are obligate intracellular, non-motile, Gram-negative bacteria of the family *Anaplasmataceae* (class *Alphaproteobacteria*: order *Rickettsiales*). The genus includes *Anaplasma phagocytophilum, A. marginale, A. centrale*, *A. bovis*, *A. ovis*, *A. platys*, *A. caudatum*, *A. odocoilei*, *A. capra* and *A. mesaenterum* (*incertaesedis*). Anaplasmae are parasites of the cells of the haematopoietic system which can persist for a long-time in infected host populations. Isolation of agents was done from ruminants, wild ruminants, pets, horses, and arthropods especially from tick vectors such as *Dermacentor, Rhipicephalus, Ixodes, Hyalomma,* and *Argas.* The negative economic impact of anaplsmosis on international animal trade and livestock production is significant. Concernning public health *A. phagocytophilum* (Silaghi et al., 2017), *A. ovis* (Chochlakis et al., 2010) and *A. capra* (Li et al., 2015a) infections are wellknown zoonoses. Deaths were reported after blood transfusions with Anaplasmae contamination (Goel et al., 2018). CDC reports show a significant increase in the incidence of human cases in the US from 384 cases in 2000 to 4,151 cases in 2016.(CDC, 2019). In addition, the increasing number of reports of infected blood donors in transfusion centers of Rabat, Morocco (Elhamiani Khatat et al., 2016) show that Anaplasmae are an emerging risk.

1. This work focuses on data of Arab countries of the North African-West Asian corridor, which is a bridge between 3 continents and acts as an important route of disease spread to Europe. A detailed analysis of the scientific literature, official reports and online media was done to comprehensively describe the current situation and risks.
2. The first chapter provides an overview of the epidemiological situation (eg common diag nostic techniques, prevalence, risk factors, etc.) for each country. The role of complicated trade relations and the impact of the biggest celebration of Muslims, Eid al-Adha in Saudi Arabia, for the spread of agents is highlighted.
3. The second chapter focuses on the situation in Egypt. The prevalence of bovine anaplasmosis was assessed by available commercial *Anaplasma* cELISA and real time PCR. The presence of homology of the recombinant protein msp5 of *Anaplasma marginale* used in the cELISA was investigated to verify possible cross reactions to other pathogens. Statistical analyzes for risk factors were done.
4. In the third chapter the attempt to optimize the commercial competitive *Anaplasma* ELISA v2 (cELISA) for use in camels' sera is described.

5. Appendix A provides some information on the cross-reactivity of the recombinant protein Msp5, which was determined by in-sillico analysis.

References

CDC 2019. Anaplasmosis: Epidemiology and Statistics.

Chochlakis, D., Ioannou, I., Tselentis, Y., Psaroulaki, A., 2010. Human anaplasmosis and *Anaplasma ovis* variant. Emerging infectious diseases 16, 1031-1032.

Dantas-Torres, F., 2015. Climate change, biodiversity, ticks and tick-borne diseases: The butterfly effect. International journal for parasitology. Parasites and wildlife 4, 452-461.

Elhamiani Khatat, S., Sahibi, H., Hing, M., Alaoui Moustain, I., El Amri, H., Benajiba, M., Kachani, M., Duchateau, L., Daminet, S., 2016. Human exposure to *Anaplasma phagocytophilum* in two cities of Northwestern Morocco. PLoS One 11, e0160880.

Goel, R., Westblade, L.F., Kessler, D.A., Sfeir, M., Slavinski, S., Backenson, B., Gebhardt, L., Kane, K., Laurence, J., Scherr, D., Bussel, J., Dumler, J.S., Cushing, M.M., 2018. Death from transfusion-transmitted anaplasmosis, New York, USA, 2017. Emerging infectious diseases 24, 1548-1550.

Li, H., Zheng, Y.-C., Ma, L., Jia, N., Jiang, B.-G., Jiang, R.-R., Huo, Q.-B., Wang, Y.-W., Liu, H.-B., Chu, Y.-L., Song, Y.-D., Yao, N.-N., Sun, T., Zeng, F.-Y., Dumler, J.S., Jiang, J.-F., Cao, W.-C., 2015. Human infection with a novel tick-borne Anaplasma species in China: a surveillance study. The Lancet Infectious Diseases 15, 663-670.

Silaghi, C., Santos, A.S., Gomes, J., Christova, I., Matei, I.A., Walder, G., Domingos, A., Bell-Sakyi, L., Sprong, H., von Loewenich, F.D., Oteo, J.A., de la Fuente, J., Dumler, J.S., 2017. Guidelines for the direct detection of *Anaplasma* spp. in diagnosis and epidemiological studies. Vector Borne Zoonotic Dis 17, 12-22.

C H A P T E R 1

Review of Literature

Overview on Anaplasmosis in Arab Countries

Partially published as:

Retrospective study of anaplasmosis in countries of North Africa and the Middle East. *Scientific and Technical Review. 2021; 39 (3). Published 2021 Mai.*

Published with the permission of the
World Organisation for Animal Health (OIE).

Summary

Anaplasmosis, also known as tick-born fever, is a zoonotic disease caused by bacteria of the genus *Anaplasma*. It affects livestock (cattle, small ruminants, camels) in tropical and sub-tropical countries worldwide. Infected animals suffer from various disorders of the he molymphatic and immune system. The economic impact of the disease is noticeable and caused by losses due to weight loss, abortions or death. The objective of this review is to provide comprehensive information on anaplasmosis in animal populations of Arabian countries in North Africa and the Middle East as categorized by the UN, which include Algeria, Egypt, Libya, Morocco, Sudan, Tunisia and Western Sahara in Africa. Others are Bahrain, Iraq, Jordan, Kuwait, Lebanon, Oman, Qatar, Syrian Arab Republic, Saudi Arabia, State of Palestine, United Arab Emirates and Yemen in Asia. For this review, relevant information from national and international scientific publications on serologic and molecular investigations was collected to evaluate the epidemiology of the disease in the time-period from 1959 to 2019. The prominent *Anaplasma* species (*Anaplasma phagocytophilum, A. marginale, A. centrale, A. bovis, A. ovis, A. platys*) currently circulating in these countries is illustrated herein a map. The animal import product share in Arabian states between 1991 and 2017 indicated the possible transmission of anaplasmosis among the countries in the corridor. Cluster analysis of deposited sequence data of the NCBI database showed distribution of similar pathogens in the study area which may be associated with animal trade during the huge animal movements, especially silent carriers across this corridor for sacrifice in famous Islamic celebration festival 'Eid al-Adha'. The spread of anaplasmosis during this celebration has not been considered in any scientific work, as done for viral infections. This is of particular interest considering the role of Saudi Arabia as a special hub for the corridor of North Africa and the Middle East and the center of Islamic world. Molecular assays indicated samples positivity of *Anaplasma* species in cattle (3.5- 69.3%), in small ruminants (2.5-95%), in camels (17.7-88.89%) and in dogs (5.4-24.4) of North African countries and 95% of cattle, 15.5- 66.7% of small ruminants, 28-95.5% of camels and 1.6-39.5% of dogs in Middle East. Serologic analysis showed seropositivity of 13.5-89.7% in cattle and 29.2% in dogs of North Africa and 35-36% of cattle, 44.7-94% in small ruminants, 10.83% in camels and 9.9% in dogs of Middle East countries. The prominent *Anaplasma* species were identified in western part of North Africa (Algeria, Morocco and Tunisia). This study revealed that anaplasmosis remains a threat not only for the economics of Arabian countries but to public health. Therefore, information monitoring and data extraction are the most important tools to optimize future control strategic programs.

Keywords: Anaplasmosis, North Africa and Middle East, comprehensive data, Regional/ Intercontinental.

Abbreviation: World Organization for Animal Health, OIE; World Health Organization, WHO; Centers for Disease Control and Prevention, CDC; International Union of Microbiological

Societies, IUMS; World Bank Group, WBG; competitive ELISA, cELISA; Arab Organization for Agricultural Development, AOAD; World Integrated Trade Solution, WITS; United Nations, UN.

Introduction

North Africa and the Middle East with predominantly hot desert or hot semi-arid climate usually face an increase of extreme heat, aridity and drought caused by climate change [1; 2]. This phenomenon has crucial impact on the agricultural and livestock production in many Arab countries, which makes significant contribution to the national economies for the countries in these regions [1; 3]. A short description on climate, landscape, population distribution etc. of each country is given in Table 1.1 [4]. Sir Arnold Theiler first described anaplasmosis in 1910. *Anaplasma* species is the causative organism. Members of the genus *Anaplasma* (*α Proteobacteria: Rickettsiales: Anaplasmataceae: Anaplasma*) are obligate intracellular, non-motile, polymorph and Gram-negative bacteria. Species of this genus are the well-recognized species *Anaplasma phagocytophilum A. marginale, A. centrale, A. bovis, A. ovis, A. platys* [5; 6] and *A. caudatum* [7]. The recently identified species are *A. odocoilei* [8]*, A. capra* [9] and *A. mesaenterum* (*incertae sedis*) [10]. Most *Anaplasma* spp. are distributed worldwide in tropical and subtropical regions. The infection cycle of the agents and their distribution are influenced by demographic, environmental and social factors such as international travel and trade or unplanned urbanization. Environmental aspects like land structure, habitat fragmentation and climate have proven influence on survival rates and may strengthen the resistance mechanisms of the agent. Other important factors are the number of available hosts, presence and questing behavior of vectors e.g. *Dermacentor, Rhipicephalus, Ixodes, Hyalomma* and *Argas* spp, tick-host encounter rate, duration of the blood meal, duration of infection of the ticks, vector competence of the ticks, efficiency of transstadial transmission, and migration of birds and livestock movements. The plethora of these elements can influence transmission, resistance and virulence of *Anaplasma* spp., or can lead to outbreaks in countries regarded as free of anaplasmosis [11-13]. The bacteria are transmitted by bites of infected ticks or flies [14], iatrogen [15], transplacental [16], and may be spread by animal migration [13] or vectors e.g. birds [17; 18]. In humans, transmission via blood transfusion and organ transplants had been documented and could lead to death [19]. Khatat et al. reported that 22% of blood donors were infected in Rabat's (Morocco) Regional Transfusion Centre [20]. Affected animals show fever, inappetence, loss of coordination, breathlessness, reduced growth rate, abortions, stillbirths and death [21]. Congenital infection has been noted [22]. Members of genus are specialized to hematopoietic and bone marrow cells, and can proliferate (replicate) in a unique, intracellular membrane-bound compartment that helps the organism to survive [16; 23; 24]. *A. phagocytophilum* has the ability to manipulate the host cell [23; 24]. Typical signs of an infection are hematological abnormalities e.g. progresssive anemia, thrombocytopenia followed by more prolonged neutropenia and lymphocytopenia (Table 1.2). A classic

postmortem finding is splenomegaly with sub-capsular bleedings and hepatomegaly with distended gallbladder [25]. Anaplasmosis can cause significant economic losses and negatively affects animal welfare and public health [26; 27]. As an example, the northeastern United States recorded a significant increase of four times in human cases of anaplasmosis from 2010 to 2017 [26]. Available data from literature imply that anaplasmosis is considered as one of the major constraints of livestock management in endemic areas and poses a hard challenge to smallholders facing reduced animal productivity. The control of this disease is costly and difficult and needs availability of diagnostic methods such as cELISA, nested PCR and real time PCR. *In silico* methods, genomics, proteomics, metagenomics, and transcriptomics analyzes [28] have been used to improve detection and to characterize genes for molecular diagnosis like 16S rRNA, groEL, msp4, Ank, and p44/msp2. These genes were targeted and PCRs have been developed [29]. Serological diagnostic methods such as cELISA and IFAT are available, but is hampered sometimes by 2-4 weeks delayed seroconversions [30]. Table 1.3 lists the relevant methods that have been used for research or diagnosis of *Anaplasma* infections so far. The Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [15] recommends cELISA for surveillance of bovine anaplasmosis. It contributes to eradication and demonstrates population freedom from infection (with regular monitoring). Real time PCR and nested PCR are used for confirmation of clinical cases and for individual animal freedom from infection before movement. Microscopic examination of blood or organ smears is also recommended for conformation of clinical cases. Despite the fact that new diagnostic methods are available, isolation of *Anaplasma* spp. is still a challenge. It requires inoculation of yolk sacs of embryonated eggs and experimental animals (mice and calf). Also, cell culture is still used. A biosafety level 2 laboratory is mandatory [31-34]. The best specimens for culture/isolation and molecular screening are whole blood and buffy coat samples. In case of a chronic disease spleen, liver, lung, lymph nodes and bone marrow are the preferred sample materials [35]. Because of long-term ability of infection in part of infected animal populations (carrier animals), regular vaccination programs for farms are required [24]. Live vaccines derived from *A. centrale* are available in several countries. This vaccine leads to partial protection within 6-8 weeks against *A. marginale*, which can last for several years after a single vaccination [15]. Tick vaccine is known e.g. BM86 for control of *A. marginale*, but varying efficacies against *Rhipicephalus* demonstrated the need for vaccine improvement [36]. Tetracyclines (tetracycline, oxytetracycline, chlortetracycline, minocycline, doxycycline, etc.) have been suggested for animal treatments [10; 16]. World Health Organization (WHO) reports stress that changing of behavior is demanded as the key issue in control of vector-borne diseases i.e. improving awareness of know-how to protect and prevent the disease [37]. Arabian countries remain a regional and international corridor for travelers of Islamic countries to celebrate “Eid al-Adha” in Saudi Arabia with a deep impact for the distribution of diseases.

The animal movement for this feast is impressive and usually disrupts veterian public health structures in the area(s). Data from animal import into this corridor are displayed as bar chart in figure 1.1. Figure 1.2 illustrates distribution of similar pathogens, by cluster analysis of deposited sequences at the NCBI database using UPGMA algorithm (BioNumerics® 10.2.3 Biomatters Ltd.) that obviously reflect trade relationships. Super-national cooperation to curtail spread of anaplasmosis in these regions is long overdue. Therefore, comprehensive epidemiological studies would be useful to clarify the course of disease, prognosis of distribution condition and establish effective plans for the future. This work presented a comprehensive overview of available information on anaplasmosis in Arabian countries of North Africa and the Middle East for evaluation of the current epidemiologic situation of this disease, at the portal to Europe. The aim of this study is to summarize the information and prepared a comprehensive overview on epidemiology of surveillance of diagnostic methods used for anaplasmosis in North Africa and the Middle East.

Materials and methods

Availability of data and data analysis

Literatures from 1959 to 2019 available from relevant databases (PubMed, Google and Science Direct) were reviewed using the search items “prevalence of anaplasmosis”, “diagnostic methods”, “risk factors” and “history of agent” for each country. Additionally, online Web-based resources (e.g. WBG, WITS, AOAD, etc) searched national and international publications. Furthermore, information on animal populations was listed (Table 1.4). Countries involved in this study are Algeria, Egypt, Libya, Morocco, Sudan, Tunisia and Western Sahara (known as North Africa) and Middle East countries such as Bahrain, Iraq, Jordan, Kuwait, Lebanon, Oman, Qatar, Syrian Arab Republic, Saudi Arabia, State of Palestine, United Arab Emirates, and Yemen categorized by UN as Western Asia. This categorization was established by the United Nations to gain a greater homogeneity for statistical convenience on population size, live circumstances etc. [38]. Data for cluster analysis were selected from NCBI. BioNumerics was used to illustrate the relation between species in regions using UPGMA algorithm. Analysis was done for separated *Anaplasma* species setting options ‘standard’, ‘Gap penalty equal zero’ and ‘Juckes & Canter correction’ in similarity coefficient panel. In cluster analysis panel UPGMA and enable degeneracy handling were checked. In ‘enable degeneracy handling’, the criterions ‘most identical matches’ and ‘Clustering+ Secondary criterion’ were selected. The international trade chart was created for the corridor based on World Integrated Trade Solution (WITS) [39] https://wits.worldbank.org/CountryProfile/en/Country//MEA/Year/2016/TradeFlow/Import/Partner/all/Product/01-05_Animal using the option partner names ‘Middle East & North Africa’ as follow. In the opening page, after click on country/region button ‘by Indicator’ option in change

selection panel was chosen. Then, these settings in change selection panel were followed; <Indicator: import product share; View by: product; Reporter country/region: name of country; Year range: select the basis; Product: animal; Partner: by country and region>. After updating the page, country-wise data in row partner names 'Middle East & North Africa' were used to illustrate the diagram.

Legislation

Verification of reliability and validity of data was done by using the standard operation procedures (SOPs) of the *Terrestrial Animal Health Code* (hereafter *Terrestrial Code*) as an international accepted document of reference [15; 40]. The main critical point was the variation of epidemiological analysis based on microscopic examination, as this technique is not approved by the OIE. However, nonspecific serological techniques were used as species-specific detection of *Anaplasma*. In these cases, based on the critical infrastructures of the healthcare system, this work also attempts to use these data with corrections as follows: 1. Studies that did not use specificity methods were corrected e.g. Microscopy, cELISA, and/ or nomenclatures were based on the possibility of geological imaging of species or hosts. 2. These corrections were done with deletion of the species by using the common name. For example *A. marginal* replaced with *Anaplasma* spp. 3. These corrected studies were highlighted with asterisks in tables 1.5-1.7. Seroprevalences were calculated as percentage of positive samples in total samples for this work.

Results

General findings

Animal populations in these countries have a steadily increasing number of susceptible hosts (Table 1.4) [41]. As a rule, animal production contributes significantly to the national economies and social welfare of smallholders families independent on the surplus generated [42]. No country has implemented monitoring or surveillance system for anaplasmosis at national level. Comprehensive studies for anaplasmosis in ticks exist only for Egypt. Reports were available from nineteen countries except Libya, Western Sahara, Bahrain, Kuwait, Lebanon, Oman, Syrian Arab Republic, and United Arab Emirates. Many researches on surveillance were done in cooperation with OIE/WHO reference laboratories, national laboratories or universities of France, Italy, Germany, Japan, UK, USA etc. The most often used diagnostics are PCR, ELISA and IFA (Tables 1.5-1.8). Detailed information on the molecular methods can be found in table 1.9. The accessible commercial serological kits used in publications are '*Anaplasma* antibody competitive ELISA v2 (VMRD Inc. Pullman, USA)', 'SNAP® 4Dx® Plus test (IDEXX; Hoofddorp, The Netherlands', '*Anaplasma* immuneglobulin G ELISA (IgG): a semi-quantitative indirect IFA (Fuller, USA)', 'indirect ELISA *A. marginale*-Ab (Svanova Biotech AB, Sweden)'

and '*A. phagocytophilum* indirect immunofluorescence test kit (Fuller Laboratories, Fullerton, CA, USA)'. The distribution of *Anaplasma* spp. for each country is shown in figure 1.3. In ten countries, laboratories tried to differentiate *Anaplasma* species while there were no data on *Anaplasma* spp in nine countries. No attempts to grow the microorganisms in cell culture was published from any of the countries in this region. Many articles applied statistical methods using statistical software. Many articles (n=22) provided data or significant information on risk factors [43-45] (Tables 1.5-1.8). GPS coordinate of the accessible articles was listed in table 1.10. Epi demi ology of anaplasmosis is described for each country as follows:

Countries of North Africa

Algeria: There are no accessible official reports at national level available. It is not clear when the agent appeared first. No evidence for a program to control or monitor the infection at national level was identified. Six scientific papers [45-50] were published in the last decade only, all of them were conducted in collaboration with international organizations such as International Centre for Agricultural Research in the Dry Areas (URMITE), International Centre for Agricultural Research in the Dry Areas (ICARDA) or French Ministry of Agriculture (DGER). Screening of sera were in place in the following cities: Batna, Béjaïa, Setif district, El Eulma, Anaba, El Tarf, Tizi Ouzou and Souk Ahras in known endemic areas. A comprehensive study on prevalent species is missing. However, of 3/6 published articles, [45; 48; 50] reported *Anaplasma* in cattle only, two in dogs [47; 49], and one in both, sheep and goats [46]. Molecular based methods such as real-time and nested-PCR were used for species identification by five of six authors [46-50]. IFA, the relevant indirect technique for the diagnosis of *Anaplasma*, showed prevalences of up to 47.7% in blood samples in dogs [47]. Only one report on microscopic detection of *Anaplasma* was available [45]. In Northern Algeria, 22.7-52% of *Rhipicephalus bursa* and *R. turanicus* ticks were infected with *A. ovis* using PCR [46]. *A. ovis* was diagnosed using PCR in serum samples of goats and sheep (54.4-61.7%) [46]. In cattle, *A. phagocytophilum* and *A. platys* [48] were detected and *A. marginale* (4.4%)*, A. centrale* (39.4%)*,* and *A. bovis* (11.1%) were found in blood samples [50]. Furthermore, four groups [45-50] presented phylogenic trees of *Anaplasma*. Three scientific papers [48-50] reported one or more of the following factors i.e. ticks species and infection, sex, sampling site, activity of animals, origin of animals, age, co-infections, governorate and type of breeding system as risk factors predisposing *Anaplasma* infections (Tables 1.5, 1.6 and 1.9). In three articles the coordinates of sample collections sites were mentioned [45; 46; 48]. The major livestock with more than 20 million heads are sheep (Table 1.4).

Egypt: Anaplasmosis is mentioned in the national report of 1966 gained from the Central Agency for Public Mobilization and Statistics, Egypt [51]. Since then, incidence of the disease has been reported in some parts of the country every year, which is reflected also in fifteen

scientific papers (Tables 5 and 7). Nevertheless, there is a lack of regular monitoring and countermeasure programs in the field. Many of these articles were published in national journals and some of those (5/15) were done in co-operation with institutions from Japan, Germany and USA [52-56]. From fifteen publications, seven reports are on cattle, 1 on water buffaloes and cattle, 1 on water buffaloes and ticks, 1 on camels, 4 on arthropods and 1 on humans. Comprehensive studies of *Anaplasma* were carried out on arthropods in 2006 [54; 55]. Scientific papers are limited to a few governorates i.e. Matrouh, Damitta, Dakahlia, Qalyubia and Qena. *A. marginale* is most often reported and confirmed in cattle [52; 57], camels [58], buffaloes [59] and arthropods such as ticks from various host animal species [54]. Frequently used diagnostic methods were conventional PCR [43; 55; 57-63], cELISA [53; 63], IFA [64; 65] and microscopy on blood samples [52; 58; 63-66]. Sequencing of *A. phagocytophilum* was reported in 2012 [61]. In 2011 and 2012 *A. phagocytophilum* was reported in 7.5% of farmers in the Nile Delta, [62] and in 13.7% of *Rhipicephalus saguineus* [61]. In a report by Loftis et al. [54] screening of ticks *Hyalomma anatolicum, anatolicum, H. anatolicum excavatum, H. dromedarii, H. impeltatum, H. marginatum rufipes, unidentified nymphal Hyalomma, Rhipicephalus (Boophilus) annulatus, R. sanguineus, R. turanicus* from 12 rural towns was conducted [54]. High positivity of *A. marginale* on tick samples of camels and buffaloes were reported between 2016 and 2017 [59; 60]. Screening of 987 fleas of the species *Xenopsylla cheopis* from 17 cities was negative for *Anaplasma* spp. using PCR technique [54]. Microscopic examination was positive in 6.3-76.9% and 59.3% in bovine and buffaloe samples, respectively (Table 5). Serological assays revealed 28-78.1% of samples positivity in cattle and buffaloes using ELISA [53; 63]. This range was between 18.8% and 61.2% in cattle using IFA [64; 65]. Only one publication reported phylogenetic tree and GPS coordinates [61]. Some works were supported by European Union (ENPI-Joint operational Programme of the Mediterranean Basin-IEVP-CT) [58], African Union/Interafrican Bureau for Animal Resources (AU-IBAR) [64], National Research Center for Protozoan Diseases of Japan [53], Friedrich-Loeffler institute of Germany and Ludwig-Maximilians-Universität (LMU), Munich [52].

Morocco: The first scientific report on the appearance of anaplasmosis was in 1998 [67]. Most of the publications were published in the last decade. A comprehensive study of the disease for the entire country is missing. The major animal population is small ruminants with more than 25 million heads. Different diagnostic methods were reported for *Anaplasma* identification by nine scientific publications [20; 68-75]. For example: four publications reported PCR as the sole diagnostic method [69; 71; 74; 75], 1 publication each used PCR and microscopy [70], PCR and ELISA [68], ELISA [73], IFA [20], and PCR, IFA and ELISA [68], respectively. Stain used in Microscopy was May-Grünwald-Giemsa (MGG) staining. Ati Lbacha et al. [70] reported 71% positive sheep blood samples collected from eleven provinces in the North of Morocco

using PCR [70]. Molecular evidence exists for *A. marginale* in blood samples i.e. 21.90% of cattle [68], candidatus *Anaplasma camelii* 39.62% of camels [71] and for *A. platy* in 7.5% of canine samples [72]. Sequence analysis confirmed *A. marginale, A. platy* and *Wolbachia* in ticks [75]. *Anaplasma*-like organism was detected in *Ixodes ricinus* in 2005 [74]. Serological methods such as competitive ELISA showed a seroprevalence of 16.5-22.8% in bovines [68; 69; 73]. IFA positive titer values (1:64 and 1:128) for *A. phagocytophilum* were found in military/ police dog handlers and blood donors i.e. 37% and 27% and 36% and 22%, respectively [20]. In addition, Elhamiani reported 7/10 and 21.9% positive samples of dog owners and dogs investigated using IFA and ELISA, respectively [72]. Phylogenetic tree work was carried out in two studies [71; 74]. Most of the scientific reports were done in cooperation with working groups of at least one of the following countries and/or organizations: Belgium [69; 72], Belgium and USA [20; 68; 73], France [71; 74], and WHO Collaborative Center for Rickettsial Diseases and Other Arthropod-borne Bacterial Diseases [75].

Sudan: According to World Bank and the Sudan Ministry of Agriculture, 30-35% of Sudan's GDP and 80% of non-oil exports were generated by livestock industries in 2016. It is the main source of income for 65% of the population, especially for poor rural families [42]. Despite the prominent role of domestic animals in Sudanese economy, there are no official reports or data on monitoring programs and countermeasures against anaplasmosis. Only eight studies were published [42; 76-83] , 5 of them deal with bovines [42; 76; 79-82], 1 with sheep [83], 1 with donkeys [77] and 1 with dogs [78]. The most commonly used technique was ELISA revealing a prevalence of 37.8-57.6% in bovines [79-82]. The nested PCR proved the presence of *A. marginale* in bovines (6.1%) [76] and in sheep (41.7%) [83] , and *A. platy* in dogs (24.4%) [78]. Ibrahim et al. [77] reported *Anaplasma* spp. in donkeys. The collaboration institutions were from Portugal and USA [76], UK [80] and Germany [82; 83]. The GPS information of sample origins were reported in three articles [76; 81; 82].

Tunisia: In Tunisia, the presence of all six *Anaplasma* species was reported in thirteen scientific articles [44; 74; 84-94]. A comprehensive epidemiological study at national level on anaplasmosis does not exist. Reports on the existence of monitoring programs and countermeasures at national level were not accessible. Scientific analyzes of 14 articles revealed 4 articles on small ruminants [88; 91; 93; 95], 2 on cattle [87; 92], 2 on both, cattle and small ruminants [85; 86], 2 on camels [44; 94], 2 on horses [84; 89], 1 on dogs [90] and 2 on ticks [74; 89]. A variety of methods was used to diagnose anaplasmosis including LAMP-, nested-, hemi nested-PCR, RFLP [85; 86; 88; 89; 93], duplex PCR [87] or duplex real time PCR [92; 94], and IFA [44; 84; 89; 90]. IFA showed the presence of *A. phagocytophilum* in camels (29.2%), horses (16.3-67%) [84; 89] and dogs (25.2%) [90]. Molecular assays confirmed a prevalence of 24.7-25.4% of *A. marginale* [87; 92], 0.6-13% of *A. phagocytophilum*

[87], 15.1% of *A. centrale* and 3.9% of *A. bovis* [92] in bovines. In small ruminants, *A. ovis* was found in 65.3-69.6% [93], *A. bovis* in 23.8-42.7% [88] and *A. platy*-like organism in 11-22.8% [85] in sheep and goat samples. *A. phagocytophilum* was demonstrated in horses (13%) and *Hyalomma marginatum* (2.3%) [89]. *A. phagocytophilum*-like organisms were present in sheep (3.9-7.7%) and goats (2.5-47.5%) [86; 93]. Presence of *A. phagocytophilum* in *Hyalomma detritum* from bovines and *I. ricinus* from environment, and *A. platys* in *R. sanguineus* from dogs were proved [74]. Alt hough, LAMP and RFLP techniques are not recommended by OIE, these methods may be of benefit for developing countries in the future. The six of thirteen published studies were created in collaboration with institutions from Italy and Spain [84-86; 93; 94]. In seven studies, sample coordination sites were clearly defined [86; 89; 90; 92-95]. Six articles made gene compareson displayed as phylogenetic trees [85; 86; 88; 92-94].

***Libya*:** The data on anaplasmosis from this country were not available and/ or accessible.

Western Sahara: A disputed territory partially occupied by Morocco has no documented and/or accessible data on anaplasmosis.

Arabian countries of the Middle East

The lack of information on anaplasmosis for Middle East countries is more obvious than for North African countries. In any of these countries, no comprehensive studies or available data exist on monitoring programs or on countermeasures against anaplasmosis at the national level.

Iraq: *Anaplasma* was first mentioned by Khayyat and Gilder in 1947 [83]. Half of the studies (3/6) were published in national journals. PCR [83] and reverse-line blotting (RLB) [96] showed 66.7% and 62.6% of sheep infected with *A. ovis* [83; 96], and ELISA [97; 98] revealed 10.83% and 35% of camel and cattle samples positivity for *Anaplasma* spp, [97; 98] respectively. However, the use of bovine ELISA kit 'indirect ELISA *A. marginale*-Ab (Svanova Biotech AB, Sweden)' on camels [97] was done without validation. Therefore, it is possible that the results were calculated slightly less or greater depending on the cut-off value and immuneglobulin defect in camels. Microscopic examination revealed a range of 4.8-21.99% positive samples in sheep [99; 100]. Although, NCBI possessed the sequences of *A. phagocytophilum*, *A. marginal*, *A. ovis*, and *A. centrale* deposited by the Iraqi universities of Baghdad Al-Qadisiyah and Al-Qasim. There were no accessible scientific papers from these studies to link with NCBI database. Consequently, only *A. ovis* was displayed in figure 1.3. The cooperation partner for 2/6 articles was from Germany, Italy, Portugal and Turkey [83; 96].

Jordan: Qablan et al. reported 39.5% *A. phagocytophilum* positive carcasses of stray dogs examined by species-specific PCR for the first time in Jordan [101]. Serological assays

revealed 9.9% infection on *A. phagocytophilum* in dogs [102]. In addition, 36%, 94% and 94% of cattle, sheep and goats were infected with *Anaplasma* spp.[103], respectively.

Qatar: Specifically, 1.6% of samples from domestic animals (dogs) were tested positive for *A. platy* using conventional PCR through the cooperation of Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Fundação para a Ciência e a Tecnologia (FCT) and Bari university in Italy [104].

Saudi Arabia: The most commonly used diagnostic methods was microscopy (4/8) [105-108] . Other methods were PCR (2/8) [109; 110], PCR and ELISA (1/8) [111] and PCR, IFA and ELISA (1/8) [112]. Three studies investigated on small ruminants, 2 on camels [106; 110], 1 on cattle [105], 1 on sheep and cattle [107] and 1 on cattle, camel, sheep, fox and spiny-tailed dabb lizards [109]. Sabana et al. [112] showed that 47.4% and 54.4% samples from small ruminants were positive using c-ELISA and IFA, respectively. Molecular investigation revealed *A. ovis* (25.3%, 15.5%) and *A. phagocytophilum* (38.1%, 20.8%) in sheep and goats, respectively [112]. The presence of *Anaplasma* in samples from slaughter animals was confirmed using a species-specific PCR [111]. *A. phagocytophilum* (36.8%) and *A. ovis* (25.3%) were found in small ruminants [111] and *A. platy*-like (30%) in camels [110]. Six studies were conducted in col laboration with South African [110] and Egyptian institutions [106; 108; 109; 111; 112].

***State of Palestine*:** A pilot study in 2015 was performed using molecular assays to screening of 723 tick samples of genera *Rhipicephalus*, *Haemaphysalis* and *Hyalomma* from the West Bank, which revealed infections of 6.5% (47/723) of *Anaplasma* spp., 2.48% on *A. ovis* and 1.79% on *A. platys* [113]. In addition, 40.4% (19/47) and 9.62% (13/135) of sheep and dogs blood samples were infected with *Anaplasma* spp. and *A. platys*, respectively [113]. Furthermore, Ravi et al reported detection of A. ovis on sheep ticks [114]. Phylogenetic analysis was done in report of Zaid et al. [113]. Both studies were conducted in collaboration with universities of UK [114] and Ireland [113]

Yemen: The only investigation accessible was done in 1987 using serology. There were no positive sample found. A paper on tick species was published in 1959 by Hoogstral and Kaiser and was interpreted by MacCartan as first published results about potential vectors of anaplasmosis [115].

Bahrain, Lebanon, Syrian Arab Republic, Oman, Kuwait and United Arab Emirates: The data on anaplasmosis from these countries were not available and/ or accessible.

Conclusion

Data monitoring and information extraction for anaplasmosis for countries of the intercomtinental region of North Africa and the Middle East was done by reviewing available literature published between 1959 and 2019. Anaplasmosis is endemic in North African countries, but no data are available and/or accessible for Libya and the disputed territory of Western Sahara. The presence of almost all economically relevant species of the genus *Anaplasma* (*A. marginale*, *A. centrale*, *A. bovis*, *A. ovis*, *A. platys* and *A. phagocytophilum*) was confirmed by species-specific PCR. Currently, *A. ovis, A. phagocytophilum* and *A. platy* have been identified using molecular techniques on samples from Iraq, Jordan, and Saudi Arabia. In countries south of the Persian Gulf, *A. phagocytophilum* was demonstrated in pets. The number of anaplasmosis positive samples using molecular assays ranged from 4.4-61.7% for cattle and sheep in Algeria, 14.08-67.37% for cattle in Egypt, 7.5- 71% for dogs and small ruminants in Morocco, 0.6-69.6% for cattle and goats in Tunisia and 6.1-24.4% for cattle and dogs in Sudan. In Middle East countries, the molecular proof of *Anaplasma* using PCR analysis indicated 62.6% posi tive sheep in Iraq, 39.5% positive dogs in Jordan, 1.6% positive dogs in Qatar, 9.6-40.4% positive dogs and sheep in State of Palestine and 15.5-38.1% positive small ruminants in Saudi Arabia. In addition, in Saudi Arabia a high number of anaplasmosis positive samples from slaughtered sheep and goats was noticed. This is of particular interest considering the role of Saudi Arabia as a special hub for the corridor of North Africa and the Middle East and the center of Islamic world. During the famous festive celebration 'Eid al-Adha' huge animal movement, especially silent carriers can cross this corridor. The seroprevalence of 47.7% of dogs in Algeria was noticed, 28-78.1% of samples of cattle and buffaloes in Egypt, 8.8-22.8% of cattle in Morocco, 16.2-67% of horses in Tunisia, 37.8-57.6% of cattle in Sudan, 10.83% of camels in Iraq and 9.9-94% of dogs, sheep and goats in Jordan were tested positive. Specifically, 22% of blood donor samples from Morocco were diagnosed positive using IFA.

Generally, accessible data imply that even in countries where the disease is well known, there are no monitoring programs for anaplasmosis. Geographical data show that the potential for spreading diseases is limited due to desert and climate. Thus, control of disease would be likely, if more attention would be paid to the role of the silent carriers. International support can obviously facilitate better control and monitoring during massive animal transports. It is obvious that further research is required on the epidemiology of anaplasmosis in the countries of the Middle East and North Africa to prevent the spread of infection to neighboring European countries.

Acknowledgements:

German Federal Foreign Office as part of the 'German biosecurity program' funded this work.

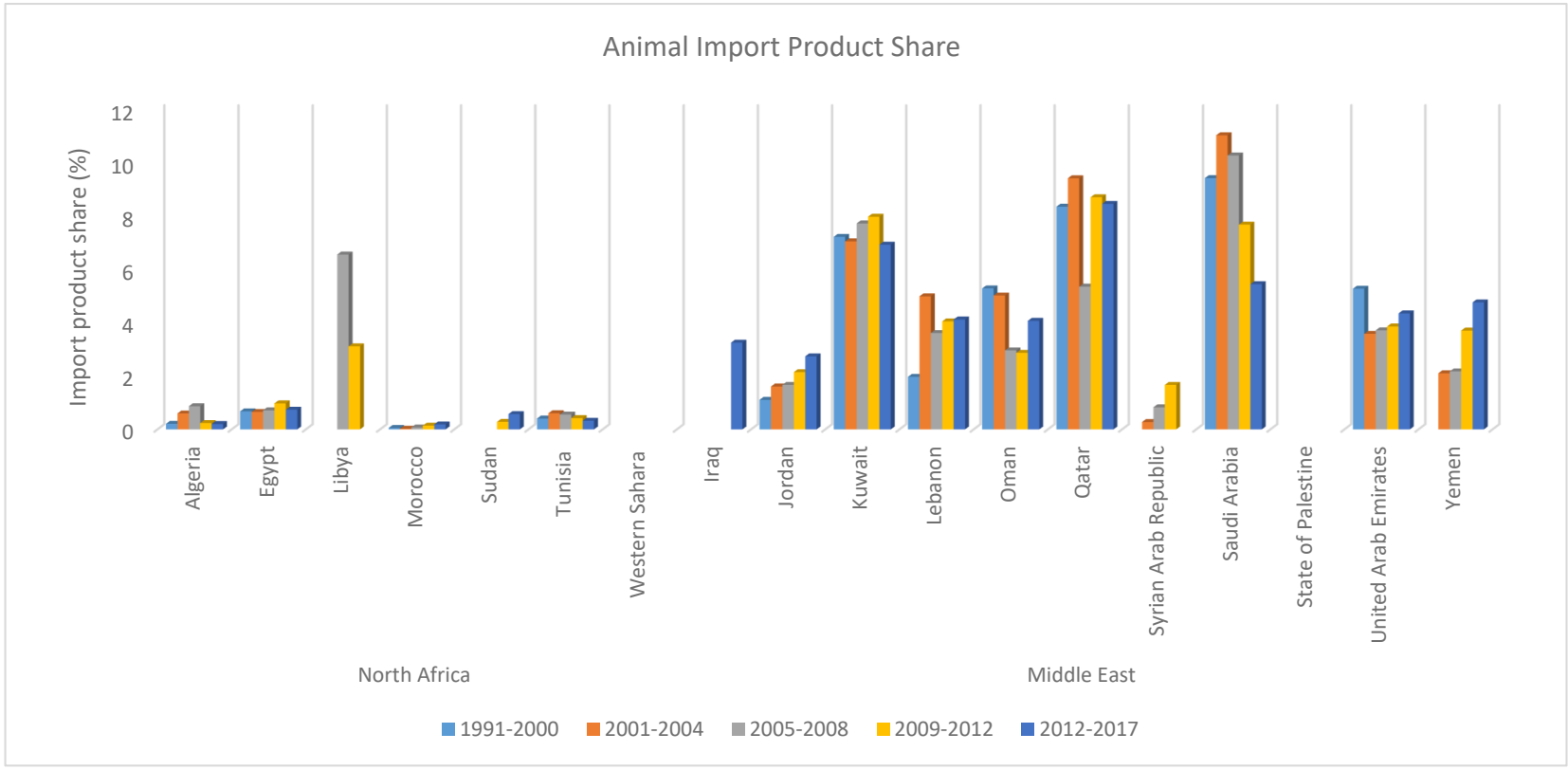

Figure 1.1. Trade relationship in the regions between 1991 and 2017 based on World Integrated Trade Solution (WITS) [39].

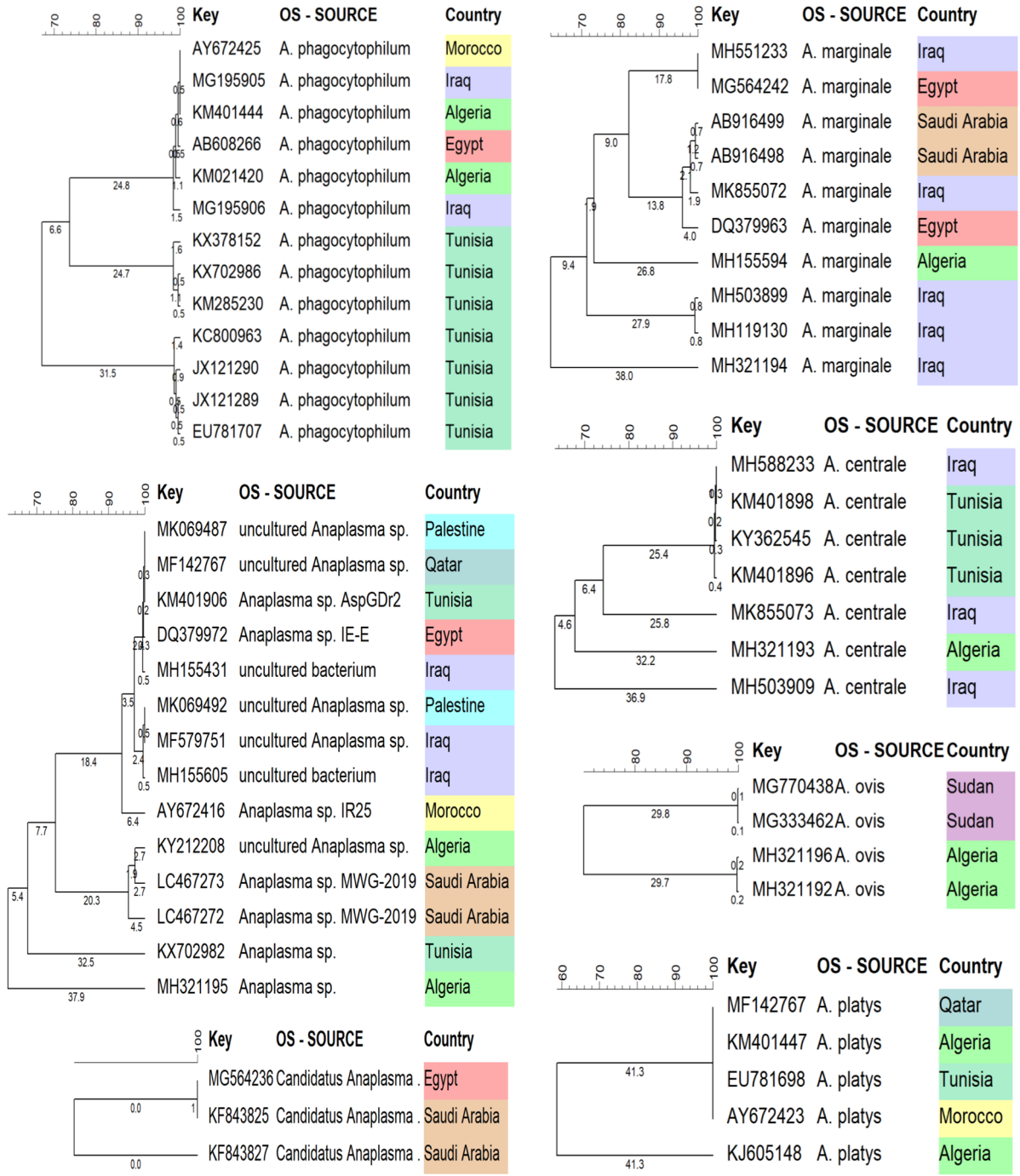

Figure 1.2. Analysis using UPGMA to show the genes homology/similarity of Anaplasma *species from Arabian countries on two continents. In case of Qatar, sequence was used as* A. *platys* and uncultured Anaplasma *sp. .*

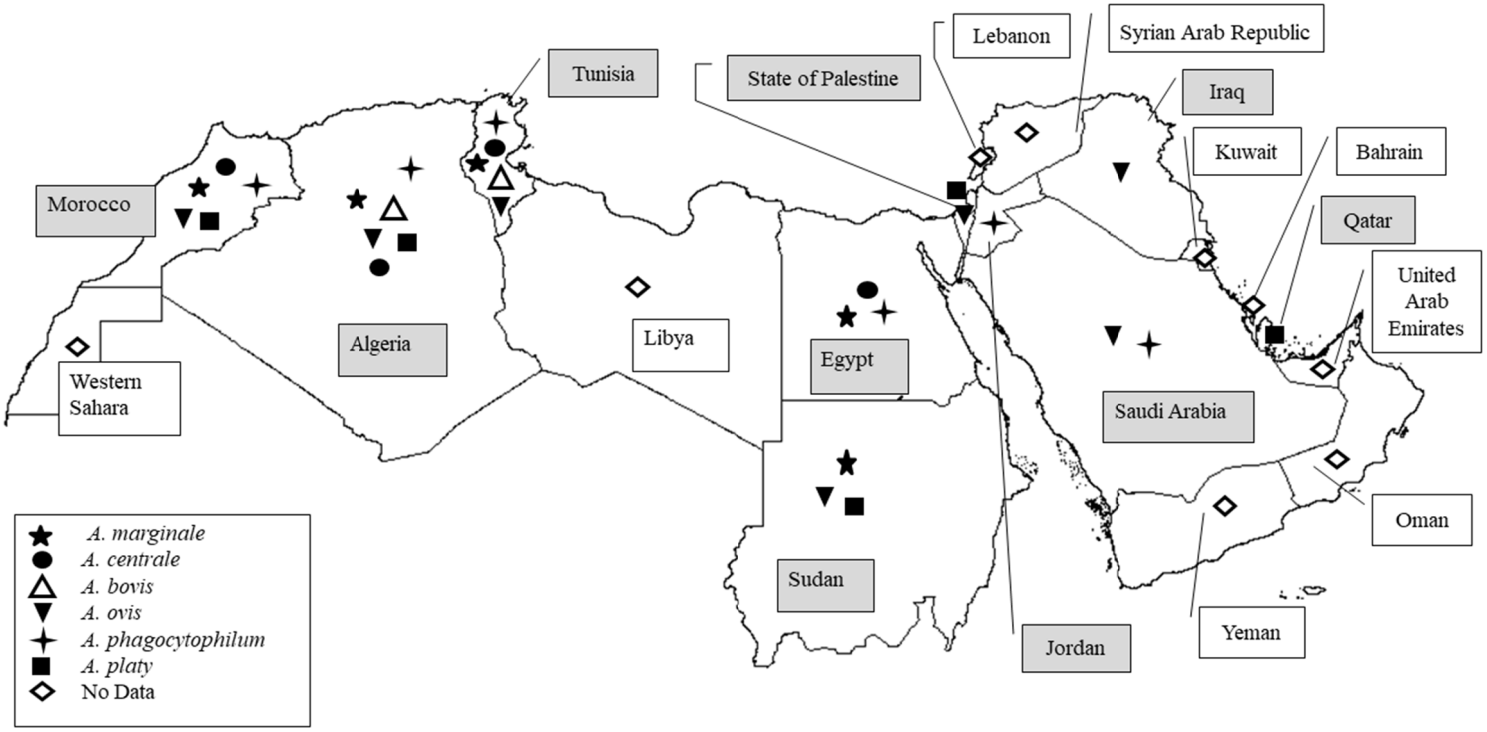

Figure 1.3. *Current distribution of anaplasmosis in countries of North Africa and the Middle East. The gray color showed the countries where* Anaplasma *spp. are present (the map is based on data from this review).*

Table 1.1. *Important facts on countries of North Africa and the Middle East [4]*

Country	Climate	Terrain	Land use			Distribution of human population	Natural hazards
			Agricultural				
			Proportion to Country	Arable	Forest		
Algeria	arid to semiarid; *coast*- winter mild, wet Summer: hot, dry *high plateau*- winter: drier-cold, Summer: hot *Sirocco*- hot, dust/sand-laden, summer wind	mainly high plateau and desert some mountains, coastal plain, narrow	17.4%	18.02%	0.82%	Vast majority in the north of country along the Mediterranean Coast	Droughts, earthquakes, floods in rainy season
Egypt	Desert, Summer: hot, dry Winter: moderate	desert interrupted by Nile	3.6%	2.8%	0.1%	Almost 95% live within 20 km of the Nile River	Droughts, earthquakes, flash floods, dust storms; sandstorms, windstorms (khamsin in spring)
Libya	Extreme desert, Mediterranean along coast	flat to undulating plains	8.8%	1%	0.1%	90% live along the Mediterranean coast	Dry, hot, dust-laden ghibli (southern wind lasting one to four days in spring), sandstorms, dust storms
Morocco	Mediterranean	-Rif Mountains (northern coast) -Atlas Mountains -large plateaus	67.5%	17.5%	11.5%	Along the Atlantic and Mediterranean coasts	Earthquakes, droughts, flash floods, windstorms
Sudan	-Hot and dry -Arid desert -Rainy season (April to November)	flat, featureless plain, desert in north	100%	15.7%	0%	Banks of the Nile, near to border with South Sudan, around Khartoum, southeast between the Blue and White Nile Rivers	Dust storms, droughts
Tunisia	-North: mild, rainy winters and hot, dry summers -South: desert	-mountains in the North; -hot, dry central plain; -semiarid south merges into the Sahara	64.8%	18.3%;	6.6%	Mostly in north	Earthquakes, droughts, flooding
Western Sahara	-Hot, dry desert -Rrain is rare; -Cold offshore air (fog and heavy dew)	flat desert with large areas of rocky or sandy surfaces rising to small mountains in south and northeast	18.8%	2.7%	0%	Mainly lives in the two-thirds of the area west of the berm (Moroccan-occupied), about 40% in Laayoune	Widespread harmattan haze (60% of time)
Baharin	-Arid, Summer: hot and humid Winter: mild	desert	11.3%	2.1%	0.7%	Northern around Manama and Al Muharraq	Dust storms, droughts
Iraq	-Desert; mild to cool winters with dry, hot, cloudless summers -Northern mountainous regions: occasionally heavy snows	mainly broad plains, reedy marshes, mountains along borders with Iran and Turkey	18.1%	8.4%	1.9%	-North, center, and Eastern. along Tigris and Euphrates Rivers	Dust storms; sandstorms; floods
Jordan	Arid desert, rainy season in west (November to April)	-desert plateau in East -highland area in West -Great Rift Valley	11.4%	2%	1.1%	Mostly in west, northwest, and southwest along the shore of the Gulf of Aqaba	Droughts, earthquakes, flash floods
Kuwait	Desert, hot summers, short, cool winters	flat to desert plain	8.5%	0.6%	0.4%	Along the Persian Gulf, (Kuwait City and on Bub iyan Island), southern half of the country	sudden cloudbursts (October to April), sandstorms and dust storms
Lebanon	-Mediterranean; -Mild to cool, wet winters with hot, dry summers; - In the mountains snows	narrow coastal plain El Beqaa (Bekaa Valley)	63.3%	11.9%	13.4%	Mediterranean coast-Beirut, Bekaa Valley	Earthquakes; dust storms, sandstorms
Oman	Dry desert; hot, humid along coast; hot, dry interior; strong southwest summer monsoon (May to September) in far south	central desert plain, rugged mountains in north and south	4.7%	0.1%	0.0%	Most ly in Al Hagar Mountains in the north, Salalah in south;	Sandstorms, dust storms, droughts
Qatar	Arid; mild, pleasant winters; very hot, humid summer	flat and barren desert	5.6%	1.1%	0.0%	Around the capital of Doha - eastern side of the peninsula	Dust storms, sandstorms
Syrian Arab Republic	Desert *Along coast:* Hot, dry, sunny summers (June to August) and mild, rainy winters (December to February); *Damascus:* cold weather (snow or sleet)	-primarily semiarid and desert plateau -narrow coastal plain -mountains in the West	75.8%	25.4%	2.7%	-Mediterranean coast -Damascus, Aleppo (the country's largest city), and Hims (Homs); Halab, and the Euphrates River valley note	Dust storms, sandstorms, volcanism
Saudi Arabia	Harsh, dry desert with great temperature extremes	mostly sandy desert	80.7%	1.5%	0.5%	-Historically nomadic or semi-nomadic -Middle of the peninsula, from Ad Dammam in the east, through Riyadh in the interior, to Mecca-Medina in the west near the Red Sea	Dust storms, volcanism

State of Palestine	West Bank (Palestin)	-Temperate -temperature and precipitation vary with altitude	mostly rugged, dissected upland in west, flat plains descending to Jordan River Valley to the east	43.3%	7.4%	1.5%	-Central to Western half of the territory -Jewish settlements in northeast, north-central, and around Jerusalem	Droughts
	Gaza Strip	Temperate	flat to rolling, sand- and dune-covered coastal plain				In major cities, particularly Gaza City in the North	Droughts
United Arab Emirates	Desert; cooler in eastern mountains		flat, barren coastal plain, desert; mountains in east	4.6%	0.5%	3.8%	Northeast on the Musandam Peninsula; 85% live in Abu Dhabi, Dubai, and Sharjah	frequent sand and dust storms
Yemen	-Desert -West coast: hot and humid -Western mountains: temperate -Extraordinarily hot, dry, harsh desert in east		narrow coastal plain backed by flat-topped hills and rugged mountains; dissected upland desert plains in center slope into the desert interior of the Arabian Peninsula	44.5%	2.2%	1%	Mostly in Asir Mountains (part of the larger Sarawat Mountain system)-western region of the country	Sandstorms and dust storms in summer

Table 1.2. *Overview of the currently reported* Anaplasma *species globaly*

Species	Main host	Comment(s) (including related disease[s])	Vectors	Clinical signs	Geographical distribution	Infected host cells	Ref.
A. marginale	Ruminants / cattle, wild ruminants	bovine anaplasmosis	*Dermacentor*, *Rhipicephalus* (*Boophilus*)	More serious in animals older than 2 years. *A. marginale* infections mainly fatal. Fever, jaundice, and anorexia. Decrease of milk production and abortion	Tropical and subtropical regions worldwide cosmopolitan	Erythrocytes	[21] [116] [14] [16] [10] [117]
A. bovis	Cattle, rabbits / dogs and wild ruminants	bovine ehrlichiosis	*Amblyomma, Haemaphysalis, Ixodes, Rhipicephalus* (*Boophilus*)	Fluctuating fever, lymphadenophaty, depression, occasionally death	Africa, Middle East, Asia, South America / worldwide, excluding Australia	Monocytes, leukocytes	
A. centrale	Cattle	anaplasmosis	*Haemaphysalis*	Mild, inapparent disease in cattle, sheep, and goats.	Europe, Africa, America, Asia	Erythrocytes	
A. ovis	Sheep, goats	ovian anaplasmosis (usually restricted to sheep and goats)	*Ixodes, Rhipicephalus, Dermacentor, Haemaphysalis, Hyalomma*	Usually subclinical	Asia, Africa, Europe, North America / mainly tropical and subtropical regions, Mediterranean Area	Erythrocytes	
A. phagocytophilum	Ruminants	tick-borne fever	*Ixodes*, *Dermacentor*	Fever, depression, lethargy, polypnea, lower milk production (in cattle), abortion	Africa, Asia, North and South America, cosmopolitan	Granolcytes (neutrophils, eosinophil, basophils); leukocytes	
	Horses	equine granulocytotropic anaplasmosis		Fever, lethargy, anorexia, limb edema, petechiae, jaundice, and ataxia In experimental infection death within 2 days			
	Dogs, cats	canine granulocytotropic anaplasmosis		Vague and similar to canine granulocytotropic lethargy, fever, lameness and joint effosion			
	Humans	human granulocytotropic anaplasmosis		Fever, headache, anorexia, malaise, abdominal pain, epigastric pain, conjunctivitis, lymphadenopathy, jaundice, rash, confusion, and cervical lymphadenopathy.	Worldwide; cosmopolitan		
A. platys	Dogs (cats, humans, ruminants);wild canids (other wild animals)	suspected canine cyclic thrombocytopenia / infectious cyclic thrombocytopenia	*Rhipicephalus*, *Haemaphysalis*	Vague and related to clotting deficiencies thrombocytopenia; weight loss, weakness, apathy, anorexia, fever, neurological symptoms, thrombocytopenia, usually associated with anemia and leukopenia,	Americas, Middle East, Mediterranean area / Europe, Taiwan, North America; cosmopolitan	Platelets	
A. capra	Small ruminants. / wild-life		Unknown	Unknown	China		[9; 118]
	Humans		Unknown	Fever, headache, anorexia, malaise, dizziness			[117]
A. caudatum	Cattle		Unknown	Unknown	North America	Erythrocytes	[7]
A. odocoilei	White-tailed deer		Unknown	Unknown	North America	Platelets	[8]
A. mesaenterum (Incertae sedis)	Sheep		*Ixodes*, *Haemaphysalis*	Unknown	Europe		[10]

Information from various sources was separated by '/ 'symbol.

Table 1.3. *Microbiologic/ diagnostic methods used for anaplasmosis.*

Microbiological methods	**Diagnostic feature**	**Ref.**
Hematology	Anemia, throbocytopenia, neutropenia	[119]
Light microscopy (Stain May-Grünwald Giemsa)	Phenotype	
Electron microscopy	Phenotype	[120]
Immunohistochemistry	Phenotype	[121]
Complement fixation test	Identification of antibodies	[15]
Indirect Immunofluorescent antibody	Identification of antibodies	[15; 122]
Western blot	Identification of antibodies	[122]
Competitive Enzyme-Linked Immunosorbent Assay	Identification of antibodies	[15]
PCR/qPCR/nested PCR/ PCR-RFLP/ multiplex PCR/Single PCR/RLB/ LAMP-PCR	Amplification of target gene sequences	[40; 93]
Sequencing and typing analysis	Amplification of target genes	
Cultivation (IDE8, ISE6, IRE/CTVM18, HL 60)	Isolation and phenotype	[31; 32; 123; 124]
Genomics, proteomics, metabolomics, and transcriptomics analyses	Genotype, phenotype	[28]
Multilocus sequence typing, genomic	Genotype	[125]
Tiling array (subtype of microarray chips)	Genotype	[126]

Table 1.4. *Number of farm animals in countries of Northern Africa and the Middle East* [41; 127].

Country	Animal No.	2008-12	2013	2014	2015	2016
Algeria	Cattle	1423490	1909460	2049650	2149550	2081000
Egypt		4800600	4745000	4762000	4883000	5012000
Libya		193000	198000	200000	201470	213850
Morocco		2896200	3029000	3238690	3291050	3300000
Sudan		36841600	30010000	30191000	30376000	30632000
Tunisia		670820	646160	671150	680450	685790
Baharin		9440*		5500	5600	6000
Iraq		2645600	2817000	2902000	1823180	1860890
Jordan		68320	69740	78260	73600	82600
Kuwait		30280		27310	29260	24250
Lebanon		74090	78000	80500	96840	81290
Oman		343330*		366680	374020	381490
Qatar		11200*		15080	26070	27910
Saudi Arabia		450950	501000	354000	293340	361360
State of Palestine		34640	33670	42390	36730	22820
Syrian Arab Republic		1084850	1074000	1108470	864470	108380
United Arab Emirates		55440*		89510	110930	108380
Yemen		1642110*		1768000	1797000	1810210
Egypt	Buffaloes	3948250	3915000	39492600	3702000	3437000
Iraq		291750	321000	331000	194390	201640
Jordan		100	100	100	100	90
Syrian Arab Republic		6580	7410	7930	7580	7200
Algeria	Sheep	22640580	26572980	27807730	28111770	28136000
Egypt		5481600	5564000	5503000	5463000	5556000
Libya		6890000	7150000	7150000	7178320	7333820
Morocco		17770790	18438025	19230840	18509600	19870000
Sudan		46696000	39568000	39846000	40210000	40612000
Tunisia		7139450	6855520	6805680	6490160	6485640
Baharin		40600*		24200	25000	30000
Iraq		7729800	8680000	8940000	6574600	6604190
Jordan		2230460	2311100	2680300	2596000	3198930
Kuwait		439380*		628040	588620	69560
Lebanon		355070	400000	450000	365490	450810
Oman		423660*		559190	570380	581780
Qatar		272400*		510450	685420	822830
Saudi Arabia		9921800	10129000	11860000	11613280	11007970
State of Palestine		640850	730890	666490	625850	521800
Syrian Arab Republic		17843550	18062840	17858140	13700790	13809920
United Arab Emirates		1555900*		2076060	2134300	2128400
Yemen		9298000*		9688000	9810070	12011410
Algeria	Goats	4201360	4910700	5129840	5013950	4935000
Egypt		4223000	4153000	4186000	4046000	4260000
Libya		2590000	2600000	2580000	2554484	2645240
Morocco		5404340	5870000	6147225	6231386	5600000
Sudan		38260200	30984000	31029000	31227000	31481000
Tunisia		1360190	1274460	1248180	1162288	1199470
Bahrain		19000*		17020	17200	20000
Iraq		1453200	1660000	1710000	1238498	1260482
Jordan		878400	836500	857730	860700	977755
Kuwait		150896*		153391	156543	172259
Lebanon		460770	450000	550000	400302	516014
Oman		180640*		2126900	2169450	2212830
Qatar		195460*		267202	324461	363568
Saudi Arabia		3772000	3408000	3450000	3149683	2596799
State of Palestine		255230	268160	264808	219941	207647
Syrian Arab Republic		1946020	2294240	2285788	1846698	1853148
United Arab Emirates		1784562*		2182082	2225532	2244445
Yemen		9083600*		9380000	9267273	9156000
Algeria	Camels	313790	344020	354465	362265	379094

Egypt		143800	153000	158269	152518	157000
Libya		170000*		57000	56455	62125
Morocco		179400	182000	57000	57500	58000
Sudan		4611000	4773000	4792000	4792000	4830000
Tunisia		72400	73000	236500	236640	237114
Baharin		1012*		2000	2000	3000
Iraq		60600	66000	68000	67048	72408
Jordan		11410	13060	13055	13200	14610
Kuwait		8475*		9192	7718	11025
Lebanon		300	200	236	200	202
Oman		153274		247710	252660	257710
Qatar		58508*		77417	84825	91195
Saudi Arabia		822400	813200	1390000	301717	1400000
State of Palestine		1280	2060	1521	1521	1521
Syrian Arab Republic		44050	53380	58715	45610	46148
United Arab Emirates		371776*		423757	430372	443568
Yemen		424000*		460000	466555	479914
Algeria	Horses	44790	54040	42010	42366	44991
Egypt		68630	71000	75000	73000	72000
Libya		45200	46000	46500	46482	45520
Morocco		154800	157000	140000	162000	180000
Sudan		784440	829000	788510	789000	790000
Tunisia		57020	57060	57010	57073	57281
Baharin		2000*		2000	2500	3000
Iraq		48300	52000	53000	50887	49885
Jordan		2380	3000	3000	3000	2229
Kuwait		1100*		1080	1177	1213
Lebanon		3410	3650	3800	3571	3229
Oman		1154*		1400	1430	1450
Qatar		5540*		2006	8349	6411
Saudi Arabia		23000	27500	33000	28550	33731
Syrian Arab Republic		14810	15100	16469	16511	15889
United Arab Emirates		n.a.		420	421	435
Yemen		1808*		2000	2083	1961
Algeria	Mules and Donkeys	181150	165000	165110	143019	138829
Egypt		1387740	1313000	1280079	1454714	1663013
Libya		29000*		29000	28950	18753
Morocco		1384020	1397690	1393000	1370000	1317000
Sudan		921880	1042000	615638	633884	663906
Tunisia		315400	322000	323100	323482	324732
Baharin		790*		5000	5000	5500
Iraq		391000	391500	390000	389746	390010
Jordan		9130	7510	10200	8937	8320
Lebanon		19870	20000	20000	19782	19042
Oman		32980*		24000	23827	23466
Saudi Arabia		100000	100000	100000	98949	98807
State of Palestine		13580	13580	13400	13580	n.a.
Syrian Arab Republic		103090	86290	81660	80352	87320
Yemen		716420*		718000	718494	731320

*In these cases data were from 2008-2013. In case of Egypt, data from statistical yearbooks of the Central Agency for Public Mobilization and Statistics Egypt (CAPMAS) was used additionally.

Table 1.5. *Overview on the epidemiology of anaplasmosis in cattle in Northern Africa and the Middle East.*

Country	Diagnostic methods	Host [Matrix Blood(b)/Sera(s)]	Total sample no. / tested	Seroprevalence (%)	Species / other factors	Collection time	Sampling area [comment(s)]	Statistical analysis [risk factor(s)]	ref.
Algeria	Nested PCR	Cattle (b)	180	42.2	*Anaplasma*	late April and early May 2015	Northeast Algeria (Setif district, El Eulma)	Sex°, breed° and age	[50]
				39.4	*A. centrale*				
				11.1	*A. bovis*				
				Neg.	*A. phagocytophilum*				
				10	Co-infection				
	Singel-PCR			4.4	*A. marginale*				
	Microscopy		161[e] suspected	15.2	*Anaplasma.* spp.*	n.a.	Wilayates of Annaba and El Tarf, eastern Algeria	Sex, breed and age	[45]
	Real time PCR, conventional PCR		36[e]/21	5	*Anaplasma* spp.	Early 2013	Batna (Veterinary practice)	n.a.	[48]
				15	*A. phagocytophilum*				
				1	*A. platys*				
Egypt	Competitive ELISA	Cattle (s)	301/90	28, 95% CI: 19.1–38.4	*Anaplasma* spp.*	May 2014 to June 2015	Southern regions (Qena or Sohag)	Local, age, type of breeding system	[53]
	Microscopy	Cattle (b)	39[e]	30/39	*A. marginale* (Detection)	Summer seasons of 2013 and 2014	Dakahlia	Clinical sign and biochemical element: glutathione (G-SH)°	[43]
	Conventionelle PCR			Confirmation					
	Microscopy		164[e]/50	100	*Parasitemia*	Summer 2012 and 2013	Dakahlia	Clinical sign and hematology Bloody feces°	[52]
	Conventional PCR		164/71 (ill)	10/71	*A. marginale*				
			164/93 (healthy)	23/93					
			164/164	20.12[c](33/164)					
			164/71	2.81	Co-infection: *Babesia*				
	Microscopy		100 [e]	40	*A. marginale*	February 2011 to August 2011	n.a., perhaps: Kaliobia	Hematology	[66]**
	Conventional PCR		100/40	26					
	Conventional PCR		100 [e]	26	*A. marginale*	February 2011 to August 2011	Kaliobia	Hematology, biochemistry°	[57]
	Microscopy		1210[e] (ear)	6.30 (S)[1]	*A. marginale*	June 2005-June 2007	Dakahilia and Daimetta Population=5290	Seasonal dynamics, age, location, type of farm°	[64]
				34.7% (n=420) (I)[2]					
	IFA[3]		350 (veins)	42 (S)					
				61.2 (I)					
	Microscopy		136[e]	85.2 (116/136)	*A. marginale*	November 2005 to October 2006	Dakahlia and Demiatta governorates	Hemology, biochemical element, season, age and type of farms	[65]
	IFA			89.7 (122/136)					
			583 (R)[4]	54.8 (320/583)		January, April, July and October 2007			
			589 (M)[5]	18.8 (111/589)					
	Microscopy		200	37 (n=74)	*Anaplasma* spp.*	June 2006 to July 2007	Matrouh governorate	Seasonal dynamics, type of animal	[63]
		Buffaloes (b)	160	59.3 (n=95)					
	cELISA[6]	Cattle (s)	200	67 (n=134)					
		Buffaloes (s)	160	78.1 (n=125)					
	Conventional PCR	Water buffalo (b)	150	104/150=69.3	*A. marginale*	n.a., perhaps 2016-17	Giza, Qalyoubia, El-Wadi El-Gadeed and Menofia	n.a.	[59]
Morocco	cELISA	Cattle (s)	1040	20.5	*Anaplasma* spp.*	n.a.	North-western, central	Location, sex, age, breed, climate, type of farm	[73]

	cELISA	Cattle (b)	668	16.50	*Anaplasma* spp.	March and August 2005	North, central Morocco	Location, age, sex, breed, climate, seasen°	[68]
	Nested PCR			21.90	*A. marginale*				
	cELISA		1764	22.8	*Anaplasma* spp.	January to December 2005	North, west (Gharb and Doukkala)	Location, sex, age°, breed°, type of farm°	[69]
	cELISA		475	8.8	*Anaplasma* spp.	n.a.	Gharb and Haouz	n.a.	[67]
Tunisia	Duplex real-time PCR or nested PCR	Cattle (b)	232	34.9	*Anaplasma* spp.	July and December 2012	Bizerte governorate (Northern Tunisia)	Bioclimatic zone°, local°, breed°, tick infestation° and breed°	[92]
				25.4	*A. marginale*				
				15.1	*A.centrale*				
				3.9	*A. bovis*				
				0.0	*A. phagocytophilum*				
	Nested PCR and RFLP[7] assay		963/367	3.5	*A. platys*-like	May and June 2015	22 delegations in North Tunisia	Location°, animal species	[85]
			936/367	0.5	*A. phagocytophilum*-like	May and June 2015	22 delegations, five governorates	Bioclimatic zone°, location	[86]
	Duplex PCR assay		328	24.7	*A. marginale*	n.a.	Northern and central Tunisia (80 farms)	Bioclimatic zone°, location, breed	[87]
				0.6	*A. phagocytophilum/ A. marginale*				
Sudan	Indirect ELISA	Calves (b)	805	57.6	*A. marginale*	September and October 2010	South Sudan	Location° and age, (more less significant)	[79]
	ELISA	Cattle (s)	243	~50	*A. marginale*	September and October 2005	South Sudan	Location	[80]
	Hot-start PCR or Nested hot-start PCR	Cattle (b)	692	6.1	*A. marginale*	n. a.	Northern Sudan: River Nile State, Aljazirah State, Kassala State and White Nile State	Location, sex, and age	[76]
	Indirect ELISA	Cattle (s)	600	38.9	*Anaplasma* spp.	June 2001 to July 2002	15 towns in the Northern, Central, Western, and Eastern Sudan	Location, age, breed	[81]
	Indirect ELISA	Cattle (s)	150	37.8	*Anaplasma* spp.	January to December 2005	Khor Rumla,Nyaing and Gumbo	Location, age, seasons and herd	[82]
Iraq	Microscopy	Cattle (b)	100	13	*Anaplasma* spp.	n. a.	Al-Aziziyah/ wasit	Clinical sign and hematology°	[98]
	cELISA			35					
Jordan	cELISA	Cattle (b)	31	36	*Anaplasma* spp	November 2015 to May 2016	Al-Dulial and the northern highlands	n.a.	[102]
Saudi Arabia	Conventional PCR	Cattle (b)	20	95	*Anaplasma* spp.	n. a.	Taif Slaughter	n. a.	[109]
				0.0	*A. phagocytophilum*				
	Microscopy*		116	1–3.4	*Anaplasma* spp.	Years 1990 and 1991	Riyadh, Tabouk, Asir, Jazan, Eastern and Northern Frontiers	Animal species, locatoin	[107]
	Microscopy*		307	Detection (0.98)	*Anaplasma* spp	Dec 1996 to Nov 1997	Bureidah Slaughter	Seasonal dynamics, source	[105]

*Legislation. **Duplicated. °significant risk factor(s). [c]Calculated for this study. [e]Herds with a history of anaplasmosis and/or apparently ill animals. [1]Sporadic cases and small holders (S). [2]Intensive system (I). [3]Immunofluorescent assay (IFA).[4]Rural Farm (R). [5]Modern farm (M). [6] Competitive ELISA (cELISA), [7]Restriction Enzyme Fragment Length Polymorphism (RFLP). n.a.: not applicable.

Table 1.6. *Overview on the epidemiology of anaplasmosis in small ruminants in Northern Africa and the Middle East*

Country	Diagnostic methods	Host [Matrix Blood(b)/Sera(s)]	Total sample no. / tested	Seroprevalence (%)	Species / other factors	Collection time	Sampling area	Statistical analysis [°significant risk factor(s)]	ref.
Algeria	Real time PCR	Sheep & goats (b)	120	74/120= 61.7 & 65/120= 54.2	*A. ovis*	April 2014 and June 2015	Souk Ahras in the Northeastern	Tick infestation	[46]
Morocco	Microscopy*		422	88.9	*Anaplasma*	December 2012 - May 2013	North	Location°, altitude° and herd size°,	[70]
	Conventional PCR			71.8					
			422/303	Neg.	*A. phagocytophilum*				
Sudan	Conventional PCR	Sheep (b)	96	41.7	*A. ovis*	n.a.	Atbara and Kartoum	n.a.	[83]
Tunisia	Conventianal PCR	Sheep & goats (b)	1685	Annual average: 35.6 & 46	*A. ovis*	March 2014 to February 2015	Northern Tunisia; Tunis, Ariana, Bizerte, Beja and Nabeul	Seasonal dynamics°	[91]
				Annual average: 7.4 & 10.1	*A. bovis*				
	Nested PCR			Neg.	*A. phagocytophilum*				
	Nested PCR coupled with RFLP[1] assay		963/241 & 355	11 & 22.8	*A. platys*-like	May and June 2015	22 delegations in North Tunisia: Tunis, Ariana, Bizerte, Beja and Nabeul	Location°, animal species, climate	[85]
			936/241 & 355	3.9 & 2.5	*A. phagocytophilum*-like			Bioclimatic zone, location	[86]
	Microscopy*	Sheep (b)	8049	4.28	*Anaplasma* spp	n.a.	Kairouan, Central Tunisia	Clinical signs, age, climate,	[95]
	LAMP PCR[2]	Sheep & Goats (b)	563/ 260 & 303	93.8 & 65.3	*A. ovis*	Between 2011 and 2013,	Northern Tunisia (El Alia, Khetmine, Joumine, Sejnane and Amdoun)	Sex, age°, breed°, tick infestation°, host	[93]
	Nested PCR			95.0 & 69.6	*Anaplasma* spp.				
				7.7 & 47.5	*A. phagocytophilum*-like				
				42.7 & 23.8	A. *bovis*	May 2011 and May to September 2013	Bizerte governorate (El Alia and Khetmine)	Sex, age, breed°, tick infestation, host, location	[88]
Iraq	Microscopy*	Sheep (b)	632	21.99	*Anaplasma* spp.	n.a.	Baghdad, Babylon, Wasit Najaf and Karbala	Age, location°, clinical signs and hematology	[99]
	PCR-RLB		195	62.6	*A. ovis*	n.a.	Kurdistan region (Duhok, Erbil and Sulaimaniya)	n.a.	[96]
	Conventional PCR		195	66.7	*A. ovis*	n.a.	Kurdistan region	n.a.	[83]
	Microscopy*		500	4.8-8.8	*Anaplasma* spp.	September-December 2007	Kurdistan region	Location, age, hematology	[100]
Jordan	cELISA	Sheep & goats (b)	68 & 36	94 & 94	*Anaplasma* spp.	November 2015 to May 2016	Ajloun, Irbid, Jarash, Tafela, Ma'an, Karak, and Mafraq	Obortion°	[102]
Saudi Arabia	Competitive ELISA	Sheep & goats (b)	312	47.4	*Anaplasma* spp.	September 2011 - November 2012	Farm and slaughtered of Medina	Sex°, age°, origin of animal	[112]
	IFA			57.4					
	Conventianal PCR			25.3 & 15.5	*A. ovis*				
				38.1 & 20.8	*A. phagocytophilum*				
	Competitive ELISA		312	44.7%	*Anaplasma* spp	Medina	n.a.	Sex, age, origin of animal	[111]
	Conventianal PCR			43.2%					
				49/189 & 30/123	*A. ovis*				
				74/189 & 41/123	*A. phagocytophilum*				
	Conventianal PCR	Sheep (b)	50	100	*Anaplasma* spp.	n.a.	Taif Slaughter	Animal species	[109]
	Microscopy*	Sheep (b)	548	2	*Anaplasma* spp.	Years 1990 and 1991	Riyadh, Tabouk, Asir, Jazan, Eastern and Northern Frontiers	Animal species	[107]

State of Palestine	Conventianal PCR	Sheep	47	40.4	*Anaplasma* spp.	January to April, 2015	Jenin, Tubas, Tulkarm, Nablus, Jericho, Ramallah, Salfit, Bethlehem and Al-Khalil	Location, Animal species, tick species	[113]

*Legislation. **Duplicate. [e]Herds with a history of anaplasmosis and/or apparently ill animals. [1] Restriction Enzyme Fragment Length Polymorphism (RFLP). [2] Loop-mediated isothermal amplification (LAMP). [3] Reverse-line blotting. n.a.: not applicable.

Table 1.7. Comprehensive overview on the epidemiology of anaplasmosis in camels of Northern Africa and the Middle East

Country	Diagnostic methods	Host [Matrix Blood(b)/Sera(s)]	Total sample no. / tested	Seroprevalence (%)	Species / other factors	Collection time	Sampling area	Statistical analysis [°significant risk factor(s)]	ref.
Egypt	Microscopy	Camel (*Camelus dromedarius)* (b)	331	47.4	*Anaplasma* spp.	March 2012-April 2015	Northern West Coast	Sex, age°, location°	[58]
	Conventianal PCR			67.37					
				22.9	*A. marginale*				
				77.13	*A. marginale*, *A. centrale*				
				78.3			Mersa Matrouh		
				88.89			El-Negella		
				61.53			Sidi-barrany		
Morocco	Conventianal PCR	Camel (*Camelus dromedarius*) (b)	106	39.62[e]	Candidatus *anaplasma camelii*, *A. platys*	December 2013 and April 2015	Southern Morocco	location	[71]
Tunisia	Duplex real-time PCR or nested PCR	Camels (*Camelus dromedarius*) (b)	226	17.70	*Anaplasma* spp. related to *A. platys*	May to October 2009	Bouficha region; Sidi Bouzid region; Douz region	Sex°, age, tick infestation	[94]
				0.0	*A. marginale*, *A. centrale*, *A. bovis*, and *A. phagocytophilum*				
	IFA[1]	Camels (*Camelus dromedarius*) (b)	226	29.2	*A. phagocytophilum*	May to October 2009	Northern Tunisia (Sidi Bouzid, Bouficha and Douz	Region, age, sex, breed, and tick infestation	[44]
Iraq	Indirect ELISA[2]	Camels (b)	120[e]	13/120 (10.83%)	*Anaplasma* spp.	January-August 2015	Al-Najaf and Wasit	Area, sex, age, clinical signs	[97]
Saudi Arabia	Microscopy	Dromedary camels (males and females) b/fsecsl	237/96	72/96	*Anaplasma* spp.*	Riyadh and Makkah	between December 2012 and March 2014	Hematology°and biochemic° element	[108]
	Conventianal PCR	Camel (spleen)	28	30 (groEL) 28 (16S rRNA)	*A. platys*-like	Unizah	Unizah slaughter	n.a.	[110]
	Conventianal PCR	Dromedary camel (b)	44	95.5	*Anaplasma* spp.	n.a.	Taif slaughter	Animal species	[109]
	Microscopy	Camels (b)	138	23.19	*Anaplasma* spp.*	May toAugust 2011	Al-Riyadh	n.a.	[106]

*Legislation. [1]Indirect immunofluorescent assay (IFA). [e]Herds with a history of anaplasmosis and/or apparently ill animals. n.a.: not applicable.

Table 1.8. Comprehensive overview on the epidemiology of anaplasmosis in small animals in Northern Africa and the Middle East

Country	Diagnostic methods	Host [Matrix Blood(b)/Sera(s)]	Total sample no. / tested	Seroprevalence (%)	Species / other factors	Collection time	Sampling area	Statistical analysis [*significant risk factor(s)]	ref.
Algeria	Real time PCR, conventional PCR	Dogs	110	6/110 = 5.4	*A. platys* *Ehrlichia canis*, 6.3% (p)	February and March 2014	Tizi Ouzou, Béjaïa	Site, breed, sex and social activity of dog	[49]
	IFA[1]	Dogs[e]	213	47.7	*A. phagocytophilum*	July 2008 to November 2010	Teaching hospital of the Algiers Veterinary School	Origin°, age, tick infestation sex, co-infection	[47]
	Conventianal PCR			14.1	*A. platys*				
				Neg.	*A. phagocytophilum*				
Morocco	ELISA	Dogs (b)	425	21.9	*Anaplasma* spp.	December 2013 and May 2015	Rabat, Kacem, Benslimane, Temara	Sex, age, ticks exposure	[72]
	Real-time PCR			7.5	*A. platy*				
Sudan	Nested PCR	Dogs (b)	78	24.40	*A. platys*	1997 to 2000	Eastern Sudan	n.a.	[78]
Tunisia	IFA	Dogs (b)	286	25.2	*A. phagocytophilum*	June and September 2006	Bizerte, Tunis, Nabeul, Nefza, Kairouan	Climate zone°, clinical and hematological sign (More less significant 10^{-8})	[90]
Jordan	Conventianal PCR	Carcasses of stray dog (b)	45/38	39.5	*A. phagocytophilum*	February–April 2006	Northern Jordan	n.a.	[101]
	SNAP® 4Dx® Plus test	Dogs (b)	161	9.9	*A. phagocytophilum*	n.a.	Amman, Ajloun, Irbid, Jarash, and the Northern Jordan Valley	Age°, sex, breed, tick infestation	[103]
Qatar	Conventianal PCR	Dogs (b)	64	1.6	*A. platy*	Doha	March to July 2016	Age, orgine, breed, sex and life style	[104]
		Cat (b)	36	Neg.					
Saudi Arabia	Conventianal PCR	Fox (*Vulpes rueppellii*) b	5	80	*Anaplasma* spp.	n.a.	Haraj animal market at Taif	Animal species	[109]
		Spiny-tailed Dabb (b) lizards (*Uromastyx ornata*) (b)	10	100					
State of Palestine	Conventianal PCR	Dogs	135	11.1	*A. platys*	January to April, 2015	Jenin, Tubas, Tulkarm, Nablus, Jericho, Ramallah, Salfit, Bethlehem and Al-Khalil	Location, animal species and tick species	[113]

[1]Indirect immunofluorescent assay (IFA). n.a.: not applicable.

Table 1.9. *Description of the molecular methods*

Country	Diagnostic methods	Species	Brief description of the methods					Ref.
			Primer: Detection	Product (bp)	Target	Thermal profile	Annealing temperature	
	Real time PCR		TtAna-f: TGACAGCGTACCTTTTGCAT TtAna-r: TGGAGGACCGAACCTGTTAC TtAna-s: FAM-GGATTAGACCCGAAACCAAG-TAMRA Ana23S-212f: ATAAGCTGCGGGGAATTGTC	280 bp	23S rRNA	-Initial action: 95°C for 15 minute (min) -40 cycles (10 second (s) at 95°C; 1 minute (min) annealing-extension at 60°C)	60 °C for 1 min	[48] [46] [49]
	Conventional PCR-		Ana23S-908r: GTAACAGGTTCGGTCCTCCA Ana23S-753r: TGCAAAAGGTACGCTGTCAC Ehr-16S-D: GGTACCYACAGAAGAAGTCC Ehr-16S-R: TAGCACTCATCGTTTACAGC		16S rRNA	-95 °C for 15 min -40 cycles (1 min at 95°C; 1-3 min extension at 72 °C) -5 min extension at 72°C.	55/60 °C for 1 min (23S) 54°C for 30 s (16S)	
	Nested-, conventional PCR	*Anaplasma* spp.	EE-1: TCCTGGCTCAGAACGAACGCTGGCGGC EE-2: AGTCACTGACCCAACCTTAAATGGCTG	1433	16S rRNA	**Liu et al. (2012)*** -94°C for 4 min -8 cycles (30 s at 94°C; 30 s at 72°C) -28 cycles (30 s at 94°C; 30 s at 72°C)	62°C for 30 s; reduced four times by 2°C every two cycle	[50]
		A. centrale:	AC1f: CTGCTTTTAATACTGCAGGACTA AC1r: ATGCAGCACCTGTGTGAGGT	426		**Kawahara et al. (2006)*** -40 cycles (30 s at 94 °C; 1 min at 72°C)	54°C for 30 s	
		A. bovis:	AB1f: CTCGTAGCTTGCTATGAGAAC B1r: TCTCCCGGACTCCAGTCTG	551		-40 cycles (1 min at 94 °C; 1 min at 72°C)	52°C for 30 s 55°C for 1 min	
		A. phago-cytophilum:	SSAP2f: GCTGAATGTGGGGATAATTTAT SSAP2r: ATGGCTGCTTCCTTTCGGTTA	641				
	Conventional PCR		MSP45: GGGAGCTCCTATGAATTACAGAGAATTGTTTAC MSP43: CCGGATCCTTAGCTGAACAGGAATCTTGC	867	msp4	**de la Fuente, Lew, et al., 2005; de la Fuente, Naranjo, et al., 2005)***		
	Conventional PCR	*A. phago-cytophilum* *A. platys*	903f: 5'- AGTTTGACTGGAACACACCTGATC-3' 1024r: 5'- CTCGTAACCAATCTCAAGCTCAAC-3' Aplatyss: 5'-TTTGTCGTAGCTTGCTATGATAAAAATT-3' SEPas: 5'- CTTCTRTRGGTACCGTCATTATCTTCCCY-3'		msp2 16S rRNA	**Beall et al. (2008)** -95 °C for 1 min -55 cycles (15 s at 94°C; 15 s at 72 °C) -5 min extension at 72°C.	58°C for 15 s	[47]
Egypt	Conventional PCR	*A. marginale* *A. centrale*	AM-F: 5'-TTG GCA AGG CAG CAG CTT-3' AM-R: 5'-TTC CGC GAG CAT GTG CAT-3' AC316: 5'-TCCAGTAACAAGCAGTTC-3' AC716: 5'-AACCCACGCGGGCAGCTT GA-3'	95 400		-96°C for 1 min -35 cycles (15s at 96 °C;20s at 72 °C for *A. marginale* and 30s at 72 °C for *A. centrale*) -1 min at 72°C	53°C for 1 min	[52]
	Conventional PCR	*A. marginale*	MAR1bB2F: 5′-GCT CTA GCA GGT TAT GCG TC-3′ MAR1bB2R: 5′-CTG CTT GGG AGA ATG CAC CT-3 Am3: GTGGCAGACGGGTGAGTAATG A Am4: CATGTCAAGAAGTGGTAAGGT	265 160	msp1ß surface protein	-94°C for 4 min -30-40 cycles (1 min at 94 °C) -72°C for 5-7 min	57°C for 1 min	[58]
	Conventional PCR	*A. marginale*	F: 5'-GCTCTAGCAGGTTATGCGTC-3' R: 5'-CTGCTTGGGAGAATGCACCT-3'	265		-95°C for 3 min -35 cycles (30s at 94 °C; 72°C for 30s) -72°C for 7 min	57°C for 30s	[60]
	Conventional PCR	*A. marginale*	F: 5'-GTGCTACGATCGCGCCTGCT-3' R: 5'-GCCCATGCCACTTCCCACGG-3'	896	msp5	-95 °C for 5 min -35 cycles (45s at 94 °C; 45s at 72 °C) -72°C for 10 min	59°C for 1 min	[57] [66]
	Conventional PCR	*Anaplasma* spp.	E1: 5'-GGCATGTAGGCGGTTCGGTAA GTT-3' E2: 5'-CCCCACATTCAGCACTCATCG TTT A-3'	262	16S rRNA	-94 °C for 2 min -30 cycles (30s at 94 °C; 30s at 72 °C -72°C for 5 min	58°C for 30s	[61; 62]
	Real time PCR	*Anaplasma* spp.	EchSYBR-F: 5'-AACACATGCAAGTCGAACGG-3' EchSYBR-R: 5'-CCC CCG CAG GGA TTA TAC A-3'	n.a.	16S rRNA	-95 °C for 10 min -40 cycles (15s at 95 °C)	60s at 60 °C	[55]
	Conventional PCR		1733F: 5′-TGTGCTTATGGCAGACCATTTCC-3′ 3134R: 5′-TCACGGTCAACCTTTGCTTACC-3′	548	Msp1α	-94 °C for 5 min -40 cycles (30s at 94 °C; 2 min at 72 °C) -72°C for 7 min	55°C for 1 min	[59]

Country	Diagnostic methods	Species	Brief description of the methods					Ref.
			Primer: Detection	Product (bp)	Target	Thermal profile	Annealing temperature	
Morocco	Real time PCR	*A. phago-cytophilum* *A. platys*	Commercial source (IDEXX Laboratories, Inc., Westbrook, Maine, USA; test code 2824 RealPCRTM test)	n.a.	Msp2	n.a.	n.a.	[72]
	Conventional PCR	*Anaplasma* spp.	AnaplatF2: 5'-GCGTAGTCCGATTCTCCAGT-3' AnaGro712R: 5'-CCGCGATCAAACTGCATACC-3'	650	groEL	-95 °C for 8 min -35 cycles (1 min at 94 °C; 1 min at 72 °C) -72°C for 10 min	59°C for 40s	[71]
		A. phago-cytophilum	903f: 5'-AGTTTGACTGGAACACACCTGATC-3' 1024r 5'-CTCGTAACCAATCTCAAGCTCAAC-3'	122	msp2	-95 °C for 5 min -35 cycles (20s at 94 °C; 1 min at 72 °C) -72°C for 10 min	50°C for 30s	
	Conventional PCR	*Anaplasma* spp.	EHR16SD: 5'- GGTACCYACAGAAGAAGTCC-3' 5'-TAGCACTCATCGTTTACAGC-3'	364	16S rRNA	-95 °C for 8 min -35 cycles (40s at 94 °C; 1 min at 72 °C) -72°C for 10 min	52°C for 40s	[70]
		A. phago-cytophilum	903f: 5'-AGTTTGACTGGAACACACCTGATC-3' 1024r: 5'-CTCGTAACCAATCTCAAGCTCAAC-3'	122	Msp2	-95 °C for 5 min -35 cycles (20s at 94 °C; 1 min at 72 °C) -72°C for 10 min	50°C for 30s	
		Anaplasma spp.	Ehr521: 5'-TGTAGGCGGTTCGGTAAGTTAAAG-3' Ehr747: 5'-GCACTCATCGTTTACAGCGTG-3'	247	rrs	-93 °C for 1 min -35 cycles (1min at 93 °C; 30s at 72 °C)-	55°C for 1min	[74]
	Nested PCR	*A. marginale*	External f: 5'-GCATAGCCTCCGCGTCTTTC-3' External r: 5'-TCCTCGCCTTGGCCCTCAGA-3' Internal f primer: 5'-TACACGTGCCCTACCGAGTTA-3'*	457 345	msp5	-95 °C for 3 min -35 cycles (30s at 95 °C; 30s at 72 °C) -72°C for 10 min	65°C for 58s	[68]
	Conventional PCR	*Anaplasma* spp.	Ehr16SD Ehr16SR	345	rrS	n.a.	n.a.	[75]
Tunisia	Conventional PCR And nested PCR	*Anaplasma* spp.	EE1: TCCTGGCTCAGAACGAACGCTGGCGGC EE2: AGTCACTGACCCAACCTTAAATGGCTG	1433	16S rRNA	**Liu et al. (2012)*/** Conventional PCR -94°C for 4 min -8 cycles (30 s at 94°C; 30 s at 72°C) -28 cycles (30 s at 94°C; 30 s at 72°C)	62°C for 30 s; reduced four times by 2°C every two cycle 54°C for 30 s	[91]
		A. bovis	AB1f: CTCGTAGCTTGCTATGAGAAC AB1r: TCTCCCGGACTCCAGTCTG	551	msp4	**Kawahara et al. (2006)*/** nested PCR -40 cycles (30 s at 94 °C; 1 min at 72°C)		
		A. ovis	AovisMSP4Fw: TGAAGGGAGCGGGGTCATGGG AovisMSP4Rev: GAGTAATTGCAGCCAGGGACTCT	344		-40 cycles (1 min at 94 °C; 1 min at 72°C)	52°C for 30 s 55°C for 1 min	
		A. phago-cytophilum	MSP45: GGGAGCTCCTATGAATTACAGAGAATTGTTTAC MSP43: CCGGATCCTTAGCTGAACAGGAATCTTGC	852	GroEL			
			EphplgroEL-F: ATGGTATGCAGTTTGATCGC EphplgroEL-R: TCTACTCTGTCTTTGCGTTC	624				
			EphplgroEL-F: ATGGTATGCAGTTTGATCGC EphgroEL-R: TTGAGTACAGCAACACCACCGGAA	573				
	Nested PCR	*A. platys*	Outer primers: EphplgroEL-F, EphplgroEL-R Inner primer: EplgroEL-R*	515	groEL	n.a.	n.a.	[85] [86]
	Nested PCR - RFLP assay	*A. phago-cytophilum*	Outer primers: EE1 and EE2 Inner primers: SSAP2f and SSAP2r	641–642	16S rRNA			
			Outer primers: EphplgroEL-F and EphplgroEL-R Inner primers: EphplgroEL-F and EphgroEL-R	573				
	Duplex PCR	*A. marginale*	M4-OvMar-F: ATCTTTCGACGGCGCTGTG M4-Mar-R: ATGTCCTTGTAAGACTCATCAAATAGC	420	msp4	-95 °C for 15 min -40 cycles (30s at 94 °C; 90s at 72 °C)	63°C for 90s	[87]
		A. phago-cytophilum	Msp2-3 F: CCAGCGTTTAGCAAGATAAGAG Msp2-3R: GCCCAGTAACAACATCATAAGC	334	msp2	-72°C for 10 min		
	Nested PCR Loop-mediated	*Anaplasma* spp. *A. ovis*	Outer primers: EE1 and EE2 Inner primers: SSAP2f and SSAP2r	641	16S rRNA	**Liu et al. (2012)*** same as upper	n.a.	[93]
			MSP4F3 Forward outer primer: GTGTTGCACACAGATTTGCC MSP4B3 Backward outer primer: AGGCTTTTGCTTCTCCGG		msp4	**Belkahia et al. (2014)**		

Country	Diagnostic methods	Species	Brief description of the methods					Ref.
			Primer: Detection	Product (bp)	Target	Thermal profile	Annealing temperature	
	isothermal amplification (LAMP) Nested PCR	 *A. phago-cytophilum* *A. bovis*	MSP4FIP Forward inner primer (F1c + F2): GCCCCTGTAGGCTAGCTTTGTGgaattcCCCATATGTGTGTGCCGG MSP4BIP Backward inner primer (B1c + B2): TGGTGGTAGGTGGGTTCTACCAgaattcATGTGCGGGTATGTCCTTG MSP4LF Loop primer F: TGTCGACAAAGCTAGCACC MSP4LB Loop primer B: CGGACTCTTTGACGAGTCTT Outer primers: EphplgroEL-F and EphplgroEL-R Inner primers: EphplgroEL-F and EphgroEL-R EE1 and EE2 AB1f and AB1r	 573	 16S rRNA 16S rRNA			[88]
	Duplex real time PCr Singel and nested PCR	*A. marginale* *A. centrale* *Anaplasma* spp. *A. centrale* *A. bovis* *A. phago-cytophilum* *A. marginale*	AM-For: TTGGCAAGGCAGCAGCTT AM-Rev: TTCCGCGAGCATGTGCAT AM-Pbc: 6FAM-TCGGTCTAACATCTCCAGGCTTTCAT-6TAMRA AC-For: CTATACACGCTTGCATCTC AC-Rev: CGCTTTATGATGTTGATGC AC-Pbd: VIC-ATCATCATTCTTCCCCTTTACCTCGT-6TAMRA EE-1: TCCTGGCTCAGAACGAACGCTGGCGGC EE-2: AGTCACTGACCCAACCTTAAATGGCTG AC1f: CTGCTTTTAATACTGCAGGACTA AC1r: ATGCAGCACCTGTGTGAGGT AB1f: CTCGTAGCTTGCTATGAGAAC AB1r: TCTCCCGGACTCCAGTCTG SSAP2f: GCTGAATGTGGGGATAATTTAT SSAP2r: ATGGCTGCTTCCTTTCGGTTA MSP45: GGGAGCTCCTATGAATTACAGAGAATTGTTTAC MSP43: CCGGATCCTTAGCTGAACAGGAATCTTGC	95 77 1433 426 551 641 852	Msp1b groEL 16S rRNA Msp4	-95 °C for 15 min -45 cycles (1 min at 95 °C; annealing-extension 60°C for 1min) **Liu et al. (2012)*** same as upper **Kawahara et al. (2006)*** same as upper **de la Fuente et al., 2005b, 2007a,b***	 n.a.	[92] [94]
	Nested PCR	*A. phago-cytophilum*	External ge3a: 5'-CACAATGCAAGTCGAACGGATTATTC-3' ge10r: 5'-TTCCGTTAAGAAGGATCTAATCTCC- 3' Internal ge9F: 5'-AACGGATTATTCTTTATAGCTTGCT-3' ge2: 5'-GGCAGTATTAAAAGCAGCTCCAGG-3'	919 546	16S rRNA	-93 °C for 1 min; 30s at 72 °C	55°C for 5min	[89]
Sudan	Hot-start PCR or semi Nested hot-start PCR	*A. marginale-A. ovis*	MSP45: 5'-GGGAGCTCCTAT-GAATTACAGAGAATTGTTTAC-3' MSP43: 5'-CCGGATCCTTAGCTGAACAGGAATCTTGC-3'		msp4	-95 °C for 2 min 40 cycles (30s at 95 °C; 72°C for 1min)	60°C for 30s	[76]
	Nested PCR	*A. platys*	**INOKUMA, H. et al. 2003*** fD1: 5'-AGA-GTT-TGA-TCC-TGG-CTCAG-3' EHR16SR: 5'-TGA-CAC-TCATCG-TTT-ACA-GC-3' PLATYS-F: 5'-AAG-TCG-AAC-GGA-TTT-TG-TC-3' PLATYS-R: 5'-CTT-TAA-CTT-ACC-GAA-CC-3'	760	16S rRNA	n.a.	n.a.	[78]
Jordan	Conventional PCR	*A. phago-cytophilum*	LA6: 5′-GAGAGATGCTTATGGTAAGAC-3′ LA1: 5′-CGTTCAGCCATCATTGTGAC-3′	444	epank1	-94 °C for 1 min -35 cycles (30s at 94 °C; 72°C for 30s) -72°C for 5 min	lowered 2°C every 2 cycles from 62 to 56°C 30s to 54°C	[101]
Iraq	PCR Reverse-line blotting (RLB)	*Anaplasma* spp.	Commercial Taq polymerase PEQLAB, Germany ATGTGAGGATTTTATCTTTGTA GGCTTTTGCC TCTGTGT A.o.-rDNA-680s: biotin-5′- TCCGGTACTGACGCTGAGGTG			 -94 °C for 3 min -40 cycles (1 min at 94 °C; 90s at 72 °C) -72°C for 5 min	 55°C for 90s	[96]

Country	Diagnostic methods	Species	Brief description of the methods					Ref.
			Primer: Detection	Product (bp)	Target	Thermal profile	Annealing temperature	
			A.o.-rDNA-1220as: 5′–AACTGAGACGACTTTTACGGATTA					
	Conventional PCR	*A. ovis*	MSP45:5′-GGGAGCTCCTATGAATTACAGAGAATTGTTTAC-3′ MSP43: 5′-CCGGATCCTTAGCTGAACAGGAATCTTGC-3′		msp4	-94 °C for 3 min -40 cycles (30s at 94 °C; 60s at 68 °C) -72°C for 5 min	60°C for 30s	[83]
Saudi Arabia	Touchdown PCR	*Anaplasma* spp.	EHR16SD: GGTACCYACAGAAGAAGTCC EHR16SR: TAGCACTCATCGTTTACAGC pA (27F): AGAGTTTGATCCTGGCTCAG EHR16SR: TAGCACTCATCGTTTACAGC EHR16SD: GGTACCYACAGAAGAAGTCC pH (1492R): GGCTACCTTGTTACGACTT EHR16SD: GGTACCYACAGAAGAAGTCC pH (1522R): AAGGAGGTGATCCAGCCGCA ELF1: GAGTTCGACGGTAAGAAGTTCA AnaGro712R: CCGCGATCAAACTGCATACC AnaPlatF2: GCGTAGTCCGATTCTCCAGT AnaGro712R: CCGCGATCAAACTGCATACC EhrlCanF3: GACATGGCAAATGTAGTTGTAAC AnaGro712R: CCGCGATCAAACTGCATACC	345 790 1030 1060 709 650 595	16S rRNA groEL	n.a.	58-56 (×2, ×3, ×35) 57-55 (×2, ×3, ×35) 55-53 (×2, ×3, ×35) 57-55 (×2, ×3, ×35) 58-55 (×2, ×3, ×35) 55-53 (×2, ×3, ×35)	[110]
	Conventional- and nested PCR	*A. phagocytophilum*	ECC: AGAACGAACGCTGGCGGC AAG CC ECB: CGTATTACC GCG GCT GCT GGC A GE9f: AACGGATTATTCTTTATAGCT TGC T GE10r: TTCCGTTAAGAAGGATCT AAT CTC C GE9f: AACGGATTATTCTTTATAGCT TGC T GE2: GGCAGTATTAAAAGCAGCTCC AGG MAP4AP5: ATGAATTACAGAGAATTG CTTGTAGG MSP$AP3: TTAATTGAAAGCAAATCT TGCTCCTATG	450-500 919 546 849	16S rRNA msp4	-94 °C for 2 min -40 cycles (1 min at 94 °C; 30s at 72 °C) -72°C for 5 min -95 °C for 2 min -35 cycles (1 min at 94 °C; 1min at 72 °C) -72°C for 7 min -95 °C for 30s -35 cycles (1 min at 94 °C) -72°C for 5 min	55°C for 2min 55°C for 75s 55°C for 1min combined with extension	[109]
State of Palestine		*Anaplasma* spp. *A. marginale, A. centrale and A. ovis*	EHR16SR: 5'- TAGCACTCATCGTTTACAGC-3' EHR16SD: 5'-GGTACCYACAGAAGAAGTCC-3' MSP45: 5'-GGGAGCTCCTATGAATTACAGAGAATTG TTTAC-3' MSP43: 5'-CCGGATCCTTAGCTGAACAGGAATCTTGC-3'	345 851	16S rRNA msp4	**de la Fuente et al. 2003***		[113]

*Data were extracted from reference articles. * PCR was done with 2 same forward or reverse primers.

Table 1.10. *GPS cordination from publications in North Africa and Middle East*

Country	GPS cordination	Ref.
Algeria	7 °08 '- 8°37' E, 36°43'- 37°7' N	[45]
	35°33.3582' N, 6°10.4484' E	[48]
	36°17'15"N 7°57'15"E	[46]
Morocco	34°150N 6°350W, 34°130N5°420W, 34°130N5°420W, 33°530N5°330W, 33 510N7 020W	[68]
	34°01'31"N 06°50'10"W, 34°13'00"N 5°42'00"W, 33°36'44"N 7°07'16"W, 33°55'36"N 6°54'44"W	[72]
Tunisia	37°160N 9°52'E, 37°16'N 10°03'E, 37°02'N 9°39'E	[92]
	36°18'N 10°27'E, 35°0'N 9°29'E, 33°27'N 9°01'E	[94]
	36°18'N 10°27'E, 35°0'N 9°29'E, 36°73'N 9°18'E, 36°51'N, 10°11'E, 36°45'N 10°73'E	[86]
	37°16'N 10°03'E-37°16'N 9°99'E-36°92'N; 9°38'E-37°15'N 9°23'E-36°76'N 9°08'E	[93]
	37°03'29.85" N 9°14'20.80"E, 36°57'26.51"N 8°45'03.95"E, 36°46'48.97"N 8°41'13.73"E, 36°26'58.43"N 8° 26'10.59"E	[89]
	35-40N 010-06E	[95]
	36°48′ 10°10′, 36°27′ 10°44′, 36°58′ 09°05′, 35°40′ 10°06′, 37°15′, 09°48′	[90]
Sudan	16–22°N 32–35°E, 14.45–17.15°N 34–37°E, 15–30°N 20–43°E, 12–13.30°N 31.30–33.15°E	[76]
	11°78′ N 19°61′ N, 22°45′ E 37°21′ E	[81]
	4º50'N, 31º35'E	[82]

References

[1] Waha K., Krummenauer L., Adams S., Aich V., Baarsch F., Coumou D., Fader M., Hoff H., Jobbins G., Marcus R., Mengel M., Otto I.M., Perrette M., Rocha M., Robinson A. & Schleussner C.F. (2017). – Climate change impacts in the Middle East and Northern Africa (MENA) region and their implications for vulnerable population groups. *Regional Environmental Change,* **17** (6), 1623-1638. doi: 10.1007/s10113-017-1144-2.

[2] Niang I., Ruppel O.C., Abdrabo M.A., Essel A., Lennard C., Padgham J. & Urquhart P. (2014). – Africa. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change *In* Climate Change 2014 Impacts, Adaptation, and Vulnerability Part B: Regional Aspects (V.R. Barros, C.B. Field, D.J. Dokken, M.D. Mastrandrea, K.J. Mach, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L.White (eds.), ed), Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA,. 1199-1265, Web Page of the Intergovernmental Panel on Climate Change (IPCC) is an alternative reference Available at: https://www.ipcc.ch/report/ar5/wg2/ (accessed on 06.17.2019 [Last seen]).

[3] Jonsson N.N., Bock R.E. & Jorgensen W.K. (2008). – Productivity and health effects of anaplasmosis and babesiosis on Bos indicus cattle and their crosses, and the effects of differing intensity of tick control in Australia. *Vet Parasitol,* **155** (1-2), 1-9. doi: 10.1016/j.vetpar.2008.03.022.

[4] (CIA) C.I.A. (2019). – The World-Factbook. Available at: https://www.cia.gov/library/publications/the-world-factbook/ (accessed on 06.17.2019 [Last seen]).

[5] Vanstreels R.E.T., Yabsley M.J., Parsons N.J., Swanepoel L. & Pistorius P.A. (2018). – A novel candidate species of *Anaplasma* that infects avian erythrocytes. *Parasites & vectors,* **11** (1), 525. doi: 10.1186/s13071-018-3089-9.

[6] Wackett L.P. (2014). – Microbial strain collections and information. *Microbial Biotechnology,* **7** (4), 371-372. doi: 10.1111/1751-7915.12135.

[7] (IUMS) I.U.o.M.S. (1984). – Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB List No. 15. *Journal*, 355-357. issn: 0020-7713/84/070355-03$02.00/0.

[8] Tate C.M., Howerth E.W., Mead D.G., Dugan V.G., Luttrell M.P., Sahora A.I., Munderloh U.G., Davidson W.R. & Yabsley M.J. (2013). – *Anaplasma odocoilei* sp. nov. (family *Anaplasmataceae*) from white-tailed deer (*Odocoileus virginianus*). *Ticks Tick Borne Dis,* **4** (1-2), 110-119. doi: 10.1016/j.ttbdis.2012.09.005.

[9] Yang J., Liu Z., Niu Q., Mukhtar M.U., Guan G., Liu G., Luo J. & Yin H. (2018). – A novel genotype of "*Anaplasma capra*" in wildlife and its phylogenetic relationship with the human genotypes. *Emerg Microbes Infect,* **7** (1), 210. doi: 10.1038/s41426-018-0212-0.

[10] Marcondes C.B. (2017). – Anaplasmosis. *In* Arthropod Borne Diseases, Springer International Publishing Switzerland 2017 doi: 10.1007/978-3-319-13884-8.

[11] Parola P., Socolovschi C., Jeanjean L., Bitam I., Fournier P.E., Sotto A., Labauge P. & Raoult D. (2008). – Warmer weather linked to tick attack and emergence of severe rickettsioses. *PLoS Negl Trop Dis,* **2** (11), e338. doi: 10.1371/journal.pntd.0000338.

[12] Merhej V., Angelakis E., Socolovschi C. & Raoult D. (2014). – Genotyping, evolution and epidemiological findings of *Rickettsia* species. *Infection, genetics and evolution : journal*

of molecular epidemiology *and evolutionary genetics in infectious diseases,* **25**, 122-137. doi: 10.1016/j.meegid.2014.03.014.

[13] Cangi N., Gordon J.L., Bournez L., Pinarello V., Aprelon R., Huber K., Lefrancois T., Neves L., Meyer D.F. & Vachiery N. (2016). – Recombination Is a Major Driving Force of Genetic Diversity in the Anaplasmataceae *Ehrlichia ruminantium*. *Front Cell Infect Microbiol,* **6**, 111. doi: 10.3389/fcimb.2016.00111.

[14] 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. doi: 10.1016/j.vetpar.2009.09.012.

[15] OIE (2019). – Chapter 3.4.1. Bovine anaplasmosis. OIE, Paris, France. Available at: http://www.oie.int/standard-setting/terrestrial-manual/access-online/ (accessed on 06.17.2019 [Last seen]).

[16] Constable P.D., Hinchcliff K.W., Done S.H. & Grünberg W. (2017). – Chapter 11-Diseases of the Hemolymphatic and Immune Systems. *In* Veterinary Medicine (Eleventh Edition), W.B. Saunders doi: https://doi.org/10.1016/B978-0-7020-5246-0.00011-5.

[17] Socolovschi C., Reynaud P., Kernif T., Raoult D. & Parola P. (2012). – Rickettsiae of spotted fever group, *Borrelia valaisiana*, and *Coxiella burnetii* in ticks on passerine birds and mammals from the Camargue in the south of France. *Ticks Tick Borne Dis,* **3** (5-6), 355-360. doi: 10.1016/j.ttbdis.2012.10.019.

[18] Mărcuţan I.D., Sándor A.D., Mihalca A.D., Gherman C.M., Kalmár Z., D'Amico G., Dumitrache M.O. & Cozma V. (2014). – Prevalence of *Anaplasma phagocytophilum* in ticks collected from migratory birds in Danube Delta, Romania. *Parasites & vectors,* **7** (Suppl 1), P16-P16. doi: 10.1186/1756-3305-7-S1-P16.

[19] Goel R., Westblade L.F., Kessler D.A., Sfeir M., Slavinski S., Backenson B., Gebhardt L., Kane K., Laurence J., Scherr D., Bussel J., Dumler J.S. & Cushing M.M. (2018). – Death from Transfusion-Transmitted Anaplasmosis, New York, USA, 2017. *Emerging infectious diseases,* **24** (8), 1548-1550. doi: 10.3201/eid2408.172048.

[20] Elhamiani Khatat S., Sahibi H., Hing M., Alaoui Moustain I., El Amri H., Benajiba M., Kachani M., Duchateau L. & Daminet S. (2016). – Human Exposure to *Anaplasma phagocytophilum* in Two Cities of Northwestern Morocco. *PLoS One,* **11** (8), e0160880. doi: 10.1371/journal.pone.0160880.

[21] Silaghi C., Santos A.S., Gomes J., Christova I., Matei I.A., Walder G., Domingos A., Bell-Sakyi L., Sprong H., von Loewenich F.D., Oteo J.A., de la Fuente J. & Dumler J.S. (2017). – Guidelines for the Direct Detection of *Anaplasma* spp. in Diagnosis and Epidemiological Studies. *Vector Borne Zoonotic Dis,* **17** (1), 12-22. doi: 10.1089/vbz.2016.1960.

[22] Henniger T., Henniger P., Grossmann T., Distl O., Ganter M. & von Loewenich F.D. (2013). – Congenital infection with *Anaplasma phagocytophilum* in a calf in northern Germany. *Acta Vet Scand,* **55**, 38. doi: 10.1186/1751-0147-55-38.

[23] Anon. (2016). – Rickettsiales: Biology, Molecular Biology, Epidemiology, and Vaccine Development, Springer International Publishing AG 2016 doi: 10.1007/978-3-319-46859-4.

[24] Stuen S., Granquist E.G. & Silaghi C. (2013). – *Anaplasma phagocytophilum*: a widespread multi-host pathogen with highly adaptive strategies. *Front Cell Infect Microbiol,* **3**, 31. doi: 10.3389/fcimb.2013.00031.

[25] Jaswal H., Bal M.S., Singla L.D., Gupta K. & Brar A.P. (2015). – Pathological observations on clinical *Anaplasma marginale* infection in cattle. *J Parasit Dis,* **39** (3), 495-498. doi: 10.1007/s12639-013-0384-4.

[26] Waxman M., White J., Dufort E.M., Eichelman A., Stellrecht K. & Kennedy J. (2018). – 666. Human Granulocytic Anaplasmosis and Ehrlichiosis Presenting to an Upstate New York Emergency Department. *Open Forum Infectious Diseases,* **5** (suppl_1), S241-S241. doi: 10.1093/ofid/ofy210.673.

[27] Okafor C.C., Collins S.L., Daniel J.A., Harvey B., Sun X., Coetzee J.F. & Whitlock B.K. (2018). – Factors associated with Seroprevalence of *Anaplasma marginale* in Kentucky cattle. *Vet Parasitol Reg Stud Reports,* **13**, 212-219. doi: 10.1016/j.vprsr.2018.07.003.

[28] Villar M., Ayllon N., Alberdi P., Moreno A., Moreno M., Tobes R., Mateos-Hernandez L., Weisheit S., Bell-Sakyi L. & de la Fuente J. (2015). – Integrated Metabolomics, Transcriptomics and Proteomics Identifies Metabolic Pathways Affected by *Anaplasma phagocytophilum* Infection in Tick Cells. *Molecular & cellular proteomics : MCP,* **14** (12), 3154-3172. doi: 10.1074/mcp.M115.051938.

[29] Guillemi E.C., Tomassone L. & Farber M.D. (2015). – Tick-borne Rickettsiales: Molecular tools for the study of an emergent group of pathogens. *J Microbiol Methods,* **119**, 87-97. doi: 10.1016/j.mimet.2015.10.009.

[30] (CDC) (2019). – Clinical and Laboratory Diagnosis. Available at: https://www.cdc.gov/anaplasmosis/healthcare-providers/clinical-lab-diagnosis.html (accessed on 06.17.2019 [Last seen]).

[31] Silveira J.A., Silvestre B.T., Bastos C.V. & Ribeiro M.F. (2016). – Isolation and attempted cultivation of an *Anaplasma marginale* strain from Brazilian brown brocket deer (Mazama gouazoubira, Fisher, 1814) in the tick cell line IDE8. *Ticks Tick Borne Dis,* **7** (6), 1102-1108. doi: 10.1016/j.ttbdis.2016.09.001.

[32] Baeta B.A., Ribeiro C.C., Teixeira R.C., Cabezas-Cruz A., Passos L.M., Zweygarth E. & Fonseca A.H. (2015). – Characterization of two strains of *Anaplasma marginale* isolated from cattle in Rio de Janeiro, Brazil, after propagation in tick cell culture. *Ticks Tick Borne Dis,* **6** (2), 141-145. doi: 10.1016/j.ttbdis.2014.11.003.

[33] Bell-Sakyi L., Zweygarth E., Blouin E.F., Gould E.A. & Jongejan F. (2007). – Tick cell lines: tools for tick and tick-borne disease research. *Trends Parasitol,* **23** (9), 450-457. doi: 10.1016/j.pt.2007.07.009.

[34] Blas-Machado U., de la Fuente J., Blouin E.F., Almazan C., Kocan K.M. & Mysore J.V. (2007). – Experimental infection of C3H/HeJ mice with the NY18 isolate of *Anaplasma phagocytophilum*. *Veterinary pathology,* **44** (1), 64-73. doi: 10.1354/vp.44-1-64.

[35] La Scola B. & Raoult D. (1997). – Laboratory diagnosis of rickettsioses: current approaches to diagnosis of old and new rickettsial diseases. *Journal*, **35** (11), 2715-2727. Available: https://www.ncbi.nlm.nih.gov/pubmed/9350721 (accessed on 06.17.2019 [Last seen]).

[36] Merino O., Alberdi P., Pérez de la Lastra J.M. & de la Fuente J. (2013). – Tick vaccines and the control of tick-borne pathogens. **3** (30) doi: 10.3389/fcimb.2013.00030.

[37] WHO (2017). – Global vector control response 2017–2030. Available at: https://www.who.int/vector-control/publications/global-control-response/en/ (accessed on 06.17.2019 [Last seen]).

[38] (UN) U.N. (2019). – Statistics Division: Methodology: Standard country or area codes for statistical use (M49). Available at: https://unstats.un.org/unsd/methodology/m49/ (accessed on 06.17.2019 [Last seen]).

[39] WITS (2019). – Middle East & North Africa 2016 Import Partner Share: Animal. Available at: https://wits.worldbank.org/CountryProfile/en/Country/MEA/Year/2016/TradeFlow/Import/Partner/all/Product/01-05_Animal (accessed on 06.17.2019 [Last seen]).

[40] (OIE) (2019). – Veterinary products (Diagnostic tests). OIE, Paris, France. Available at: http://www.oie.int/scientific-expertise/veterinary-products (accessed on 06.17.2019 [Last seen]).

[41] AOAD (2017). – Arab Agricultural Statistics Yearbooks. Available at: http://www.aoad.org/EAASYXX.htm (accessed on 06.17.2019 [Last seen]).

[42] WBG (2019). – The World Bank Group and Sudan's Ministry of Agriculture Launch the 2016 Enabling the Business of Agriculture Report. Available at: http://www.worldbank.org/en/news/press-release/2016/05/16/the-world-bank-group-and-sudans-ministry-of-agriculture-launch-the-2016-enabling-the-business-of-agriculture-report (accessed on 06.17.2019 [Last seen]).

[43] El-Ashker M., Salama M., El-Sebaei M., Risha E., Abdelhamid F., El-Diasty M. & El-Fadle E. (2016). – Significance of clinical variables and selected biochemical markers in predicting the outcome of bovine anaplasmosis. *Veterinární Medicína,* **60** (No. 6), 301-308. doi: 10.17221/8244-vetmed.

[44] Ben Said M., Belkahia H., Sayahi L., Aloui M., Jemli M.H., Hadj Mohamed B., Sassi L., Darghouth M.A., Djaiem A.A., Bayoudh M. & Messadi L. (2014). – First serological study of the prevalence of *Anaplasma phagocytophilum* in dromedary (*Camelus dromedarius*) in Tunisia. *Bulletin de la Societe de pathologie exotique (1990),* **107** (1), 1-6. doi: 10.1007/s13149-013-0323-8.

[45] Ziam H., Ababou A., Kazadi J.M. & Berkvens D. (2016). – Prévalences et signes cliniques associés des piroplasmoses bovines dans les Wilayates d'Annaba et El Tarf, Algérie. *Journal*, **9-10**, 241-249. Available: https://www.revmedvet.com/artdes-fr.php?id=16107 (accessed on 06.17.2019 [Last seen]).

[46] Aouadi A., Leulmi H., Boucheikhchoukh M., Benakhla A., Raoult D. & Parola P. (2017). – Molecular evidence of tick-borne hemoprotozoan-parasites (*Theileria ovis* and *Babesia ovis*) and bacteria in ticks and blood from small ruminants in Northern Algeria. *Comp Immunol Microbiol Infect Dis,* **50**, 34-39. doi: 10.1016/j.cimid.2016.11.008.

[47] Azzag N., Petit E., Gandoin C., Bouillin C., Ghalmi F., Haddad N. & Boulouis H.J. (2015). – Prevalence of select vector-borne pathogens in stray and client-owned dogs from Algiers. *Comp Immunol Microbiol Infect Dis,* **38**, 1-7. doi: 10.1016/j.cimid.2015.01.001.

[48] Dahmani M., Davoust B., Benterki M.S., Fenollar F., Raoult D. & Mediannikov O. (2015). – Development of a new PCR-based assay to detect Anaplasmataceae and the first report of *Anaplasma phagocytophilum* and *Anaplasma platys* in cattle from Algeria. *Comp Immunol Microbiol Infect Dis,* **39** (Supplement C), 39-45. doi: 10.1016/j.cimid.2015.02.002.

[49] Dahmani M., Loudahi A., Mediannikov O., Fenollar F., Raoult D. & Davoust B. (2015). – Molecular detection of *Anaplasma platys* and *Ehrlichia canis* in dogs from Kabylie, Algeria. *Ticks Tick Borne Dis,* **6** (2), 198-203. doi: 10.1016/j.ttbdis.2014.12.007.

[50] Rjeibi M.R., Ayadi O., Rekik M. & Gharbi M. (2018). – Molecular survey and genetic characterization of *Anaplasma centrale*, *A. marginale* and *A. bovis* in cattle from Algeria. *Transbound Emerg Dis,* **65** (2), 456-464. doi: 10.1111/tbed.12725.

[51] CAPMAS (2015). – Animal Diseases. Available at: http://www.capmas.gov.eg/Pages/Publications.aspx?page_id=5104&Year=23381;http://www.t-series.capmas.gov.eg/agreculur.aspx; https://www.capmas.gov.eg/ (accessed on 06.17.2019 [Last seen]).

[52] El-Ashker M., Hotzel H., Gwida M., El-Beskawy M., Silaghi C. & Tomaso H. (2015). – Molecular biological identification of *Babesia*, *Theileria*, and *Anaplasma* species in cattle in Egypt using PCR assays, gene sequence analysis and a novel DNA microarray. *Vet Parasitol,* **207** (3-4), 329-334. doi: 10.1016/j.vetpar.2014.12.025.

[53] Fereig R.M., Mohamed S.G.A., Mahmoud H., AbouLaila M.R., Guswanto A., Nguyen T.T., Ahmed Mohamed A.E., Inoue N., Igarashi I. & Nishikawa Y. (2017). – Seroprevalence of *Babesia bovis*, *B. bigemina*, *Trypanosoma evansi*, and *Anaplasma marginale* antibodies in cattle in southern Egypt. *Ticks Tick Borne Dis,* **8** (1), 125-131. doi: 10.1016/j.ttbdis.2016.10.008.

[54] Loftis A.D., Reeves W.K., Szumlas D.E., Abbassy M.M., Helmy I.M., Moriarity J.R. & Dasch G.A. (2006). – Rickettsial agents in Egyptian ticks collected from domestic animals. *Exp Appl Acarol,* **40** (1), 67-81. doi: 10.1007/s10493-006-9025-2.

[55] Loftis A.D., Reeves W.K., Szumlas D.E., Abbassy M.M., Helmy I.M., Moriarity J.R. & Dasch G.A. (2006). – Surveillance of egyptian fleas for agents pf public health significance: *Anaplasma*, *bartonella*,*coxiella*, *ehrlichia*. *Journal*, **75**, 41-48. Available: https://www.ncbi.nlm.nih.gov/pubmed/16837707 (accessed on 06.17.2019 [Last seen]).

[56] Loftis A.D., Reeves W.K., Szumlas D.E., Abbassy M.M., Helmy I.M., Moriarity J.R. & Dasch G.A. (2006). – Population survey of Egyptian arthropods for rickettsial agents. *Ann N Y Acad Sci,* **1078**, 364-367. doi: 10.1196/annals.1374.072.

[57] Radwan M.E.I., Ali A.F. & Hamied O.A.e. (2013). – Epidemiological Studies ,Molecular Diagnosis of *Anaplasma marginale* in Cattle and Biochemical Changes Associated with it in Kaliobia Governorate. *American Journal of Infectious Diseases and Microbiology,* **1** (3), 46-49. doi: 10.12691/ajidm-1-3-2.

[58] El-Naga T.R.A. & Barghash S.M. (2016). – Blood Parasites in Camels (*Camelus dromedarius*) in Northern West Coast of Egypt. *Journal of Bacteriology & Parasitology,* **07** (01) doi: 10.4172/2155-9597.1000258.

[59] Elhariri M.D., Elhelw R.A., Hamza D.A. & Soliman D.E. (2017). – Molecular detection of *Anaplasma marginale* in the Egyptian water bufaloes (*Bubuloes bubalis*) based on major surface protein 1α. *Journal*, **47**, 247 - 252. Available: http://www.parasitology.eg.net/pdf/82017/2.pdf (accessed on 06.17.2019 [Last seen]).

[60] M Barghash S. & A Hafez A. (2016). – Molecular Detection of Pathogens in Ticks Infesting Camels in Matrouh Governorate, Egypt. *Journal of Bacteriology & Parasitology,* **07** (02) doi: 10.4172/2155-9597.1000269.

[61] Ghafar M.W. & Amer S.A. (2012). – Prevalence and first molecular characterization of *Anaplasma phagocytophilum*, the agent of human granulocytic anaplasmosis, in Rhipicephalus sanguineus ticks attached to dogs from Egypt. *Journal of Advanced Research,* **3** (2), 189-194. doi: 10.1016/j.jare.2011.08.002.

[62] Ghafar M.W. & Eltablawy N.A. (2011). – Molecular Survey of Five Tick-Borne Pathogens (*Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi sensu lato* and *Babesia microti*) in Egyptian Farmers. *Journal*, **7 (3)**, 249-255. Available: https://www.idosijournals.org/gv/GV7(3)11/8.pdf (accessed on 06.17.2019 [Last seen]).

[63] El-Naga T.R.A., Mahmoud. M.A., Osman. W.A. & Goda A.S.A. (2009). – Serological survey of *Anaplasma marginale* (*Rickettsia*) antibodies in animal by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. *Journal*, **IVX (1)** Available: http://vet.scuegypt.edu.eg/attach/h24.pdf.

[64] Salm F.F., Younis E.E., Hegazy N.M. & El-Sawalhy A.A. (2011). – Epidemiological studies on bovine anaplasmosis. *Journal*, **59**, 179-189. Available: https://www.ajol.info/index.php/bahpa/article/view/74418 (accessed on 06.17.2019 [Last seen]).

[65] Younis E.E., Hegazy N.A.M., El-Deeb W. & El-khatib R.M. (2009). – Epidemiological and biochemical studies on bovine anaplamosis in dakahlia and demiatta governorates in Egypt. *Journal*, **57** Available: https://www.ajol.info/index.php/bahpa/article/view/51668 (accessed on 06.17.2019 [Last seen]).

[66] Abdel Hamid O.M., Radwan M.E.I. & Ali A.F. (2014). – Biochemical Changes Associated with *Anaplasma* Infection in Cattle. *Global Journal of Biotechnology & Biochemistry,* **9 (1)**, 19-23. doi: 10.5829/idosi.gjbb.2014.9.1.8290.

[67] Sahibi H., Rhalem A., Berrag B. & Goff W.L. (1998). – Seroprevalence of bovine anaplasmosis in Morocco. *Ann N Y Acad Sci,* **849**, 427-429. doi: 10.1111/j.1749-6632.1998.tb11088.x.

[68] Ait Hamou S., Rahali T., Sahibi H., Belghyti D., Losson B., Goff W. & Rhalem A. (2012). – Molecular and serological prevalence of *Anaplasma marginale* in cattle of North Central Morocco. *Res Vet Sci,* **93** (3), 1318-1323. doi: 10.1016/j.rvsc.2012.02.016.

[69] Ait Hamou S., Rahali T., Sahibi H., Belghyti D., Losson B. & Rhalem A. (2012). – Séroprévalences des hémoparasitoses bovines dans deux régions irriguées du Maroc. *Journal*, **163**, 480-485. Available: https://www.revmedvet.com/artdes-fr.php?id=15866 (accessed on 06.17.2019 [Last seen]).

[70] Ait Lbacha H., Alali S., Zouagui Z., El Mamoun L., Rhalem A., Petit E., Haddad N., Gandoin C., Boulouis H.J. & Maillard R. (2017). – High Prevalence of *Anaplasma* spp. in Small Ruminants in Morocco. *Transbound Emerg Dis,* **64** (1), 250-263. doi: 10.1111/tbed.12366.

[71] Ait Lbacha H., 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? *Infectious diseases of poverty,* **6** (1), 1. doi: 10.1186/s40249-016-0216-8.

[72] Elhamiani Khatat S., Daminet S., Kachani M., Leutenegger C.M., Duchateau L., El Amri H., Hing M., Azrib R. & Sahibi H. (2017). – *Anaplasma* spp. in dogs and owners in north-western Morocco. *Parasites & vectors,* **10**, 202. doi: 10.1186/s13071-017-2148-y.

[73] Rahali T., Sahibi H., Sadak A., Ait Hamou S., Losson B., Goff W.L. & Rhalem A. (2014). – Séroprévalence et facteurs de risque des hémoparasitoses (theiléríose, babésiose et anaplasmose) chez les bovins dans quatre grandes régions d'élevage du Maroc. *Revue d'élevage et de médecine vétérinaire des pays tropicaux,* **67 (4)**, 235-240.

[74] Sarih M., M'Ghirbi Y., Bouattour A., Gern L., Baranton G. & Postic D. (2005). – Detection and identification of *Ehrlichia* spp. in ticks collected in Tunisia and Morocco. *Journal of clinical microbiology,* **43** (3), 1127-1132. doi: 10.1128/JCM.43.3.1127-1132.2005.

[75] Seng P., Sarih M., Socolovschi C., Boudebouch N., Hassar M., Parola P., Raoult D. & Brouqui P. (2009). – Detection of Anaplasmataceae in ticks collected in Morocco. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases,* **15**, 86-87. doi: 10.1111/j.1469-0691.2008.02251.x.

[76] Awad H., Antunes S., Galindo R.C., do Rosario V.E., de la Fuente J., Domingos A. & El Hussein A.M. (2011). – Prevalence and genetic diversity of *Babesia* and *Anaplasma* species in cattle in Sudan. *Vet Parasitol,* **181** (2-4), 146-152. doi: 10.1016/j.vetpar.2011.04.007.

[77] Ibrahim A., Geysen D., Angara T., Isamail A. & Shadia A. (2011). – Assessment of Prevalence and Molecular Characteristics of Piroplasma of Working Donkeys in Khartoum State, Sudan. *Journal*, **7** Available: https://www.ajol.info/index.php/apra/article/view/76252 (accessed on 06.18.2019 [Last seen]).

[78] Inokuma H., Oyamada M., Davoust B., Boni M., Dereure J., Bucheton B., Hammad A., Watanabe M., Itamoto K., Okuda M. & Brouqui P. (2006). – Epidemiological survey of *Ehrlichia canis* and related species infection in dogs in eastern Sudan. *Ann N Y Acad Sci,* **1078**, 461-463. doi: 10.1196/annals.1374.085.

[79] Kivaria F.M., Kapaga A.M., Mbassa G.K., Mtui P.F. & Wani R.J. (2012). – Epidemiological perspectives of ticks and tick-borne diseases in South Sudan: cross-sectional survey results. *The Onderstepoort journal of veterinary research,* **79** (1), E1-E10. doi: 10.4102/ojvr.v79i1.400.

[80] Malak A.K., Mpoke L., Banak J., Muriuki S., Skilton R.A., Odongo D., Sunter J. & Kiara H. (2012). – Prevalence of livestock diseases and their impact on livelihoods in Central Equatoria State, Southern Sudan. *Preventive veterinary medicine,* **104** (3-4), 216-223. doi: 10.1016/j.prevetmed.2011.12.001.

[81] Salih D.A., Abdel Rahman M.B., Mohammed A.S., Ahmed R., Kamal S. & El Hussein A.M. (2009). – Seroprevalence of tick-borne diseases among cattle in the Sudan. *Parasitology research,* **104** (4), 845-850. doi: 10.1007/s00436-008-1265-0.

[82] Salih D.A., Hassan S.M., Julla, II, Kyule M.N., Zessin K.H. & El Hussein A.M. (2008). – Distribution and application of ELISA for the seroprevalence of tick-borne diseases in Central Equatoria State, Sudan. *Transbound Emerg Dis,* **55** (5-6), 257-262. doi: 10.1111/j.1865-1682.2008.01032.x.

[83] Renneker S., Abdo J., Salih D.E., Karagenc T., Bilgic H., Torina A., Oliva A.G., Campos J., Kullmann B., Ahmed J. & Seitzer U. (2013). – Can Anaplasma ovis in small ruminants be neglected any longer? *Transbound Emerg Dis,* **60 Suppl 2**, 105-112. doi: 10.1111/tbed.12149.

[84] Ben Said M., Belkahia H., Heni M.M., Bouattour A., Ghorbel A., Gharbi M., Zouari A., Darghouth M.A. & Messadi L. (2014). – Seroprevalence of *Anaplasma phagocytophilum* in well maintained horses from northern Tunisia. *Tropical biomedicine,* **31** (3), 432-440.

[85] Ben Said M., Belkahia H., El Mabrouk N., Saidani M., Alberti A., Zobba R., Cherif A., Mahjoub T., Bouattour A. & Messadi L. (2017). – *Anaplasma platys*-like strains in ruminants from Tunisia. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases,* **49**, 226-233. doi: 10.1016/j.meegid.2017.01.023.

[86] Ben Said M., Belkahia H., El Mabrouk N., Saidani M., Ben Hassen M., Alberti A., Zobba R., Bouattour S., Bouattour A. & Messadi L. (2017). – Molecular typing and diagnosis

of *Anaplasma* spp. closely related to *Anaplasma phagocytophilum* in ruminants from Tunisia. *Ticks Tick Borne Dis,* **8** (3), 412-422. doi: 10.1016/j.ttbdis.2017.01.005.

[87] M'Ghirbi Y., Beji M., Oporto B., Khrouf F., Hurtado A. & Bouattour A. (2016). – *Anaplasma marginale* and *A. phagocytophilum* in cattle in Tunisia. *Parasites & vectors,* **9** (1), 556. doi: 10.1186/s13071-016-1840-7.

[88] Ben Said M., Belkahia H., Karaoud M., Bousrih M., Yahiaoui M., Daaloul-Jedidi M. & Messadi L. (2015). – First molecular survey of *Anaplasma bovis* in small ruminants from Tunisia. *Vet Microbiol,* **179** (3-4), 322-326. doi: 10.1016/j.vetmic.2015.05.022.

[89] M'Ghirbi Y., Yaich H., Ghorbel A. & Bouattour A. (2012). – *Anaplasma phagocytophilum* in horses and ticks in Tunisia. *Parasites & vectors,* **5**, 180. doi: 10.1186/1756-3305-5-180.

[90] M'Ghirbi Y., Ghorbel A., Amouri M., Nebaoui A., Haddad S. & Bouattour A. (2009). – Clinical, serological, and molecular evidence of ehrlichiosis and anaplasmosis in dogs in Tunisia. *Parasitology research,* **104** (4), 767-774. doi: 10.1007/s00436-008-1253-4.

[91] Belkahia H., Ben Said M., El Mabrouk N., Saidani M., Cherni C., Ben Hassen M., Bouattour A. & Messadi L. (2017). – Seasonal dynamics, spatial distribution and genetic analysis of *Anaplasma* species infecting small ruminants from Northern Tunisia. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases,* **54**, 66-73. doi: 10.1016/j.meegid.2017.06.016.

[92] Belkahia H., Ben Said M., Alberti A., Abdi K., Issaoui Z., Hattab D., Gharbi M. & Messadi L. (2015). – First molecular survey and novel genetic variants' identification of *Anaplasma marginale*, *A. centrale* and *A. bovis* in cattle from Tunisia. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases,* **34**, 361-371. doi: 10.1016/j.meegid.2015.06.017.

[93] Ben Said M., Belkahia H., Alberti A., Zobba R., Bousrih M., Yahiaoui M., Daaloul-Jedidi M., Mamlouk A., Gharbi M. & Messadi L. (2015). – Molecular Survey of *Anaplasma* Species in Small Ruminants Reveals the Presence of Novel Strains Closely Related to *A. phagocytophilum* in Tunisia. *Vector Borne Zoonotic Dis,* **15** (10), 580-590. doi: 10.1089/vbz.2015.1796.

[94] Belkahia H., Ben Said M., Sayahi L., Alberti A. & Messadi L. (2015). – Detection of novel strains genetically related to *Anaplasma platys* in Tunisian one-humped camels (*Camelus dromedarius*). *Journal of infection in developing countries,* **9** (10), 1117-1125. doi: 10.3855/jidc.6950.

[95] Gharbi M. (2015). – Epidemiological Study of Sheep Anaplasmosis (*Anaplasma ovis* Infection) in Kairouan, Central Tunisia. *The Journal of Advances in Parasitology,* **2** (2), 30-34. doi: 10.14737/journal.jap/2015/2.2.30.34.

[96] Renneker S., Abdo J., Bakheit M.A., Kullmann B., Beyer D., Ahmed J. & Seitzer U. (2013). – Coinfection of sheep with *Anaplasma*, *Theileria* and *Babesia* species in the Kurdistan Region, Iraq. *Transbound Emerg Dis,* **60 Suppl 2**, 113-118. doi: 10.1111/tbed.12148.

[97] Al-Gharban H.A. & AL-Taee H.S. (2016). – Serological diagnosis of *Anaplasma marginale* bacteria in carrier Arabian one- humped camels. *Journal*, **15** Available: https://www.iasj.net/iasj?func=fulltext&aId=124334 (accessed on 06.18.2019 [Last seen]).

[98] Al-Gharban H.A. & Dhahir S.H. (2014). – Serological diagnosis of persistent infection with *Anaplasma marginale* bacteria in cattle. *Journal*, **39** (1), 33 -39. Available: https://www.iasj.net/iasj?func=fulltext&aId=103628 (accessed on 08.02.2019 [Last seen]).

[99] Mohammed A.H. & Salman K.O. (2016). – Epidemiological and hematological studies of anaplasma spp. in sheep in middle part of Iraq. *Journal*, **15** Available: https://www.iasj.net/iasj?func=fulltext&aId=124315 (accessed on 06.18.2019 [Last seen]).

[100] Mustafa B.H.s. (2012). – Clinical and hematological study on ovine anaplasmosis in sulaimani province-Iraq. *Journal*, **11** Available: https://www.iasj.net/iasj?func=article&aId=55032 (accessed on 07.01.2019 [Last seen]).

[101] Qablan M.A., Kubelova M., Siroky P., Modry D. & Amr Z.S. (2012). – Stray dogs of northern Jordan as reservoirs of ticks and tick-borne hemopathogens. *Parasitology research,* **111** (1), 301-307. doi: 10.1007/s00436-012-2839-4.

[102] Obaidat M.M. & Salman A.E.B. (2019). – Anaplasma spp. in dairy ruminants in Jordan: high individual and herd-level seroprevalence and association with abortions. *J Vet Diagn Invest,* **31** (3), 481-484. doi: 10.1177/1040638719843171.

[103] Obaidat M.M. & Alshehabat M.A. (2018). – Zoonotic Anaplasma phagocytophilum, Ehrlichia canis, Dirofilaria immitis, Borrelia burgdorferi, and spotted fever group rickettsiae (SFGR) in different types of dogs. *Parasitology research,* **117** (11), 3407-3412. doi: 10.1007/s00436-018-6033-1.

[104] Alho A.M., Lima C., Latrofa M.S., Colella V., Ravagnan S., Capelli G., Madeira de Carvalho L., Cardoso L. & Otranto D. (2017). – Molecular detection of vector-borne pathogens in dogs and cats from Qatar. *Parasites & vectors,* **10** (1), 298. doi: 10.1186/s13071-017-2237-y.

[105] El-Metenawy T.M. (2000). – Prevalence of blood parasites among cattle at the central area of Saudi Arabia. *Vet Parasitol,* **87** (2-3), 231-236. doi: https://doi.org/10.1016/S0304-4017(99)00158-2.

[106] Al-Khatib R.M., Mazloum K.S. & Al Nakhli H.M. (2012). – Incidence of anaplasmosis and FMD in camel (*Camelus dromedaries*). *Journal*, **58** Available: http://www.aun.edu.eg/journal_files/76_J_8754.pdf (accessed on 06.17.2019 [Last seen]).

[107] Al-Khalifa M.S., Hussein H.S., Diab F.M. & Khalil G.M. (2009). – Blood parasites of livestock in certain Regions in Saudi Arabia. *Saudi journal of biological sciences,* **16** (2), 63-67. doi: 10.1016/j.sjbs.2009.10.002.

[108] 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. *Journal of Camel Practice and Research,* **23** (1), 95-100. doi: 10.5958/2277-8934.2016.00014.X.

[109] Ghafar M.W. & Shobrak M.Y. (2014). – Molecular detection and characterization of *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis, in some animals suspected to be competent reservoirs in Taif district, Kingdom of Saudi Arabia. *Journal*, **11** Available: https://www.semanticscholar.org/paper/Molecular-detection-and-characterization-of-the-of-Ghafar-Shobrak/5b4bb9a7904fc69737c9f8efd534a723033fa6dd (accessed on 06.18.2019 [Last seen]).

[110] 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 *Ehrlichia canis* in the dromedary camel (*Camelus dromedarius*). *Vet Microbiol,* **179** (3-4), 310-314. doi: 10.1016/j.vetmic.2015.06.001.

[111] Taha H.A., Shoman S.A. & Alhadlag N.M. (2015). – Molecular and serological survey of some haemoprotozoan, rickettsial and viral diseases of small ruminants from Al-Madinah Al Munawarah, KSA. *Journal*, **32** (3), 511-523. Available: https://www.ncbi.nlm.nih.gov/pubmed/26695213; https://www.semanticscholar.org/paper/Molecular-and-serological-survey-of-some-%2C-and-of-Munawarah-Ksa/495e9ffecc2e4685d6fc7d63455554a6b00b1f1d (accessed on 07.01.2019 [Last seen]).

[112] Shabana, II, Alhadlag N.M. & Zaraket H. (2018). – Diagnostic tools of caprine and ovine anaplasmosis: a direct comparative study. *BMC Vet Res,* **14** (1), 165. doi: 10.1186/s12917-018-1489-x.

[113] Zaid T., Ereqat S., Nasereddin A., Al-Jawabreh A., Abdelkader A. & Abdeen Z. (2019). – Molecular characterization of Anaplasma and Ehrlichia in ixodid ticks and reservoir hosts from Palestine: a pilot survey. *Vet Med Sci,* **5** (2), 230-242. doi: 10.1002/vms3.150.

[114] Ravi A., Ereqat S., Al-Jawabreh A., Abdeen Z., Abu Shamma O., Hall H., Pallen M.J. & Nasereddin A. (2019). – Metagenomic profiling of ticks: Identification of novel rickettsial genomes and detection of tick-borne canine parvovirus. *PLoS neglected tropical diseases,* **13** (1), e0006805-e0006805. doi: 10.1371/journal.pntd.0006805.

[115] Mccartan B.M., Hunter A.G., Pegram R.G. & Bourne A.S. (1987). – Tick Infestations on Livestock in the Yemen-Arab-Republic and Their Potential as Vectors of Livestock Diseases. *Tropical animal health and production,* **19** (1), 21-31. doi: Doi 10.1007/Bf02250841.

[116] Rar V. & Golovljova I. (2011). – *Anaplasma*, *Ehrlichia*, and "*Candidatus Neoehrlichia*" bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases,* **11** (8), 1842-1861. doi: 10.1016/j.meegid.2011.09.019.

[117] Li H., Zheng Y.C., Ma L., Jia N., Jiang B.G., Jiang R.R., Huo Q.B., Wang Y.W., Liu H.B., Chu Y.L., Song Y.D., Yao N.N., Sun T., Zeng F.Y., Dumler J.S., Jiang J.F. & Cao W.C. (2015). – Human infection with a novel tick-borne Anaplasma species in China: a surveillance study. *The Lancet. Infectious diseases,* **15** (6), 663-670. doi: 10.1016/S1473-3099(15)70051-4.

[118] Peng Y., Wang K., Zhao S., Yan Y., Wang H., Jing J., Jian F., Wang R., Zhang L. & Ning C. (2018). – Detection and Phylogenetic Characterization of *Anaplasma capra*: An Emerging Pathogen in Sheep and Goats in China. *Front Cell Infect Microbiol,* **8** (283), 283. doi: 10.3389/fcimb.2018.00283.

[119] Roland L., Drillich M. & Iwersen M. (2014). – Hematology as a diagnostic tool in bovine medicine. *J Vet Diagn Invest,* **26** (5), 592-598. doi: 10.1177/1040638714546490.

[120] Granquist E.G., Aleksandersen M., Bergström K., Dumler S.J., Torsteinbø W.O. & Stuen S. (2010). – A morphological and molecular study of Anaplasma phagocytophilum transmission events at the time of Ixodes ricinus tick bite. *Acta veterinaria Scandinavica,* **52** (1), 43-43. doi: 10.1186/1751-0147-52-43.

[121] Dumler J.S., Choi K.-S., Garcia-Garcia J.C., Barat N.S., Scorpio D.G., Garyu J.W., Grab D.J. & Bakken† J.S. (2005). – Human Granulocytic Anaplasmosis and

Anaplasma phagocytophilum. *Emerging infectious diseases,* **11** doi: 10.3201/eid1112.050898.

[122] Park J.H., Heo E.J., Choi K.S., Dumler J.S. & Chae J.S. (2003). – Detection of antibodies to *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* antigens in sera of Korean patients by western immunoblotting and indirect immunofluorescence assays. *Clin Diagn Lab Immunol,* **10** (6), 1059-1064. doi: 10.1128/cdli.10.6.1059-1064.2003.

[123] de la Fuente J., Garcia-Garcia J.C., Blouin E.F., Saliki J.T. & Kocan K.M. (2002). – Infection of tick cells and bovine erythrocytes with one genotype of the intracellular ehrlichia *Anaplasma marginale* excludes infection with other genotypes. *Clin Diagn Lab Immunol,* **9** (3), 658-668. doi: 10.1128/cdli.9.3.658-668.2002.

[124] Bell-Sakyi L., Palomar A.M., Bradford E.L. & Shkap V. (2015). – Propagation of the Israeli vaccine strain of *Anaplasma centrale* in tick cell lines. *Vet Microbiol,* **179** (3-4), 270-276. doi: 10.1016/j.vetmic.2015.07.008.

[125] Huhn C., Winter C., Wolfsperger T., Wuppenhorst N., Strasek Smrdel K., Skuballa J., Pfaffle M., Petney T., Silaghi C., Dyachenko V., Pantchev N., Straubinger R.K., Schaarschmidt-Kiener D., Ganter M., Aardema M.L. & von Loewenich F.D. (2014). – Analysis of the population structure of *Anaplasma phagocytophilum* using multilocus sequence typing. *PLoS One,* **9** (4), e93725. doi: 10.1371/journal.pone.0093725.

[126] Nelson C.M., Herron M.J., Felsheim R.F., Schloeder B.R., Grindle S.M., Chavez A.O., Kurtti T.J. & Munderloh U.G. (2008). – Whole genome transcription profiling of *Anaplasma phagocytophilum* in human and tick host cells by tiling array analysis. *BMC Genomics,* **9**, 364. doi: 10.1186/1471-2164-9-364.

[127] AOAD (2017). – Arab Agricultural Ministries web-Sites. Available at: http://www.aoad.org/elink1.htm (accessed on 06.17.2019 [Last seen]).

C H A P T E R 2

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Pathogens. 2020; 9(1): E64. Published 2020 Jan 16.

doi: 10.3390/pathogens9010064

pathogens

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Seroprevalence and Molecular Detection of Bovine Anaplasmosis in Egypt

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Received: 09 December 2019; Accepted: 13 January 2020; Published: date

Abstract: Bovine anaplasmosis is a tick-borne disease with zoonotic potential, caused by the obligate intracellular bacterium *Anaplasma marginale.* The disease is distributed worldwide in tropical and subtropical regions. The economic losses from anaplasmosis in animals is of significant importance because it causes severe morbidity and mortality in cattle. Recovered animals may become persistent carriers. Epidemiological information on the actual status of bovine anaplasmosis in Egypt is scarce. Thus, this study aimed to determine anti-*Anaplasma* antibody and DNA in serum samples using ELISA and PCR, respectively. In total, 758 bovine sera were collected from cattle farms located in 24 Egyptian governorates in 2015 to 2016. Sera were analyzed with the commercially available '*Anaplasma* antibody competitive ELISA v2' kit and 'AmpliTest *Anaplasma/Ehrlichia* spp. real time TaqMan ™ PCR. *Anaplasma* spp. antibodies were detected in 140 (18.5%) (CI: 15.8–21.4%) of the investigated sera by ELISA, and Anaplasma/Ehrlichia-DNA was detected in 40 (5.3%) (CI: 3.8–7.1%) of the positive sera by real time PCR. Co-detection of both *Anaplasma* spp. and *Coxiella burnetii*-specific antibodies was proven in 30 (4%) of the investigated sera. The results of this work confirm the significant prevalence of bovine anaplasmosis in Egypt. Raising awareness in decision makers of the public health, veterinarians and animal owners is required to reduce the spread of infection.

Keywords: *Anaplasma marginale*; Bovine anaplasmosis; *Coxiella burnetii*; Egypt; prevalence; ELISA; real time PCR.

1. Introduction

Bovine anaplasmosis is caused by the obligate intracellular bacterium *Anaplasma marginale*, (Alphaproteobacteria: Rickettsiales: Anaplasmataceae) that was first described by Sir Arnold Theiler in 1910 as the causative agent of gall sickness in cattle [1]. Anaplasmosis is a tick-borne disease and bacteria replicate within the epithelial cells of the tick midgut [2,3]. It is endemic in tropical and subtropical areas worldwide. Anaplasmosis could be misdiagnosed with other tick-borne diseases caused by *Babesia* (*B.*) *bovis* and *B. bigemina*, which have a similar geographical distribution and cause anemia in cattle [4]. Besides transmission by ticks, these hemoprotozoa and *A. marginale* can also be transmitted mechanically by biting flies [5], needles [6], ear-tagging, castration and dehorning equipment [7,8], and parasites of migratory wild birds [9,10].

Other *Anaplasma* species that may cause bovine anaplasmosis are *A. centrale* causing only a mild disease, and *A. bovis* and *A. phagocytophilum* known as bovine ehrlichiosis and tick-borne fever, respectively [11]. They can infect cattle and cause a reduction of milk production. Bovine congenital transmission was reported for *A. phagocytophilum* [12], which has been recognized as a zoonotic agent [8,13]. The severity of symptoms depends on several host factors such as its immune status and possible coinfections by other pathogens [13]. Symptoms occur after a latency period i.e., progressive anemia due to multiplication of *A. marginale* or *A. centrale* within mature erythrocytes. Other symptoms are fever, inappetence, loss of coordination, breathlessness, reduced growth rate, abortions, and stillbirth. Compared to other pathogenic bacteria, there is no report proving the transmission of *Anaplasma* spp. to humans via animal products [14]. In humans, blood transfusion and organ transplantation have been recognized as modes of transmission for *A. phagocytophilum* [15–17].

Anaplasma spp. in general have long life persistence and are able to remain in populations for months or years, which has a significant influence on spreading and new outbreaks of anaplasmosis [8,18,19]. Control measures should include regular monitoring, timely treatment and countermeasures against the arthropod vectors [5], but the feasibility depends on various factors such as geographic location and implementation costs of regulatory measures e.g. use of vaccines or antibiotics [20]. Variations of vector competence and limitations of our knowledge on the tick immune responses hinder control efforts and especially our understanding of the arthropod–microbe interaction [21]. Despite the limited current knowledge, a tick vaccine is already under development [22].

Bovine anaplasmosis is an economically important disease that causes losses in the dairy and beef industries through reduced milk production, weight loss, abortion, icterus, and even death in some cases [23,24]. There exists no reports on the antibiotic resistance of these pathogens. Tetracyclines and imidocarb are recommended by the World Organisation for Animal Health (OIE) to reduce probable side effects of an attenuated *A. centrale* live vaccine [6]. Marcondes reported on successful oxytetracycline treatment [25].

The NCBI database holds only two complete whole genome sequences of *A. marginale* and four of *A. phagocytophilum* isolates. Diagnostic assays used in veterinary medicine to identify *A. marginale* and *A. centrale* showed that the competitive ELISA (cELISA) test is recommend for monitoring and screening of populations while PCR and Giemsa are recommended for staining for the examination of clinical cases [6].

The average number of cattle kept per year in Egypt between 2002 and 2014 was more than 4.6 million, highlighting the importance of dairy and meat production in this country. Bovine anaplasmosis in Egypt was mentioned first in the national report of 1966 [26]. Since then, the disease was detected in many governorates. In Egypt, several studies reported anaplasmosis caused by *A. marginale* in cattle, water buffaloes and camel [27–32]. Frequently used techniques in these reports were microscopy [30], competitive ELISA (cELISA) [33,34], immunofluorescent assay (IFA) [35,36], or molecular assays i.e. conventional PCR [27] or real-time PCR [37].

Epidemiological studies are useful for the monitoring and control of diseases, and subsequently, the reduction of costs. For bovine anaplasmosis, such studies were limited to some governorates, and a comprehensive study for the whole of Egypt is missing. The objective of this study was to update the epidemiological information about bovine anaplasmosis in Egypt through investigating the prevalence of anaplasmosis in cattle within 27 Egyptian governorates using cELISA and real time PCR, to predict risk factors and provide baseline data for an effective design of disease control.

2. Materials and Methods

2.1. Study Area and Sample Information

Egypt is a vast desert plateau interrupted by the Nile valley and Delta region. Approximately 95% of the human population lives within 20 km of the Nile River and its delta. This territory is divided into 27 governorates, which have been categorized into three large domains: the Western part, the Eastern part and the Nile Valley and Delta region. In total, 758 cattle serum samples were collected during a Q

fever prevalence study between October 2015 and March 2016 from 61 different farms located in 61 districts (sample sites) of 24 governorates (North Sinai, South Sinai and Luxor were excluded). A questionnaire that contained information about the animals, such as age, husbandry systems, infesting parasites, contact with other animals (i.e., dogs, etc.), and GPS data was used in this work (Figure 2.1). Age was categorized in two groups: ≤4 or >4 years. Three different husbandry systems were present: stable/stationary, pasture and nomadic.

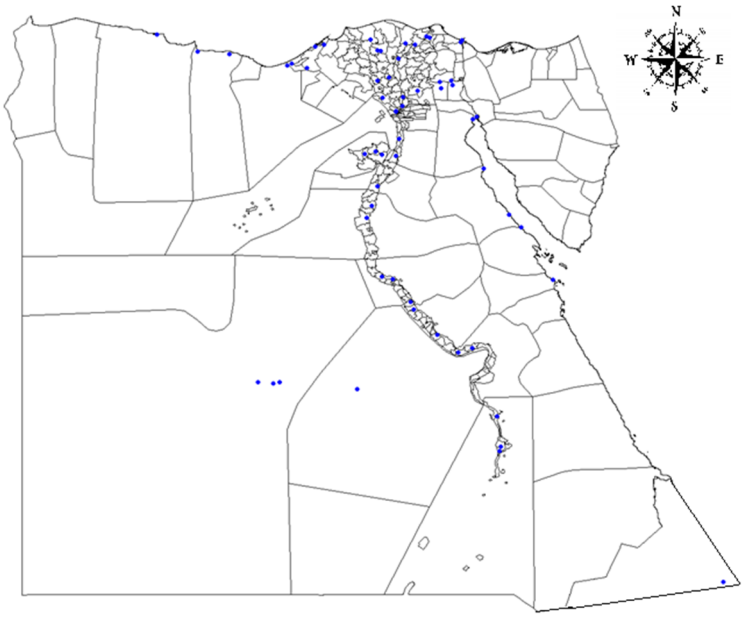

Figure 2.1. Sampling sites in Egypt. The map illustrates the position of sampling sites in each governorate.

The distribution of 758 cattle sera (Figure 2.1) was 283 (37.33%) from the Nile Delta domain, 337 (44.06%) from the Western domain, and 138 (18.2%) from the Eastern domain. Out of the 758 investigated cattle, 414 (54.61%) were kept in stables/stationary and 310 (40.89%) were nomadic. Tick infestation was recorded in 55.8% (n = 423), and 60.16% of animals were older than 4 years. All data regarding age group, animal housing and others are summarized in Table 2.1.

Table 2.1. Number of animals sampled per domain with age group, husbandry system and tick infestation.

Domain		Western Domain	Nile Delta	Eastern Domain	Total
Cattle		334 (44.06%)	283 (37.33%)	135 (18.2%)	758
Animal age	≤ 4 years	175 (57.94%)	73 (24.17%)	54 (17.81%)	302 (39.84%)
	> 4 years	162 (35.52%)	210 (46.05%)	84 (18.42%)	456 (60.16%)
Animal husbandry	Stable/Stationary	No samples	280 (67.63%)	134 (32.36%)	414 (54.61%)
	Nomadic	303 (97.74%)	3 (0.96%)	4 (1.29%)	310 (40.89%)
	Nomadic & Pasture	34	(-)	(-)	34 (4.48%)
Tick infestation		193 (45.62%)	149 (36.69%)	81 (19.14%)	423 (55.8%)
Cattle kept in spatial separate		(-)	280 (68.96%)	126 (31.03%)	406 (53.56%)
Others animal species living on farm		8 (32%)	15 (60%)	2 (8%)	25 (3.29%)

(-) No samples were available.

2.2. Detection of Anaplasma spp.-Specific Antibodies Using cELISA

Sera were stored at −20°C and tested for specific antibodies against *Anaplasma* spp. using a competitive ELISA (cELISA) (Veterinary Medical Research and Development Inc., Pullman, WA, USA) according to the manufacturer's instructions. The assay has a sensitivity of 100% and specificity of 99.7%

according to the supplier [38]. All sera were tested in duplicate. Results were calculated according to manufacturer's recommendation: percentage inhibition (% I) = 100 (1 − [sample OD620/OD620 of negative control]). Samples with a value ≥ 30% were considered as positive.

2.3. Detection of Anaplasma spp./Ehrlichia spp. DNA Using Real Time PCR

The DNA was extracted and purified from all seropositive and suspected positive samples with only one positive cELISA result using High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. The concentration and quality of DNA was measured using a NanoDrop1000® (Thermo Fisher, Wilmington, USA) according to the manufacturer's guidelines. The mean DNA concentration was 11.31 ± 6.9 ng/µL. DNA samples were stored at −20 °C until further use. For detection of *Anaplasma* spp./*Ehrlichia* spp.-specific DNA, the AmpliTest *Anaplasma* spp./*Ehrlichia* spp. Kit (Amplicon Ltd., Wrocław, Poland) was used according to the manufacturers guidelines. The assay has a sensitivity and specificity of 100% according to the manufacturer. The presence of *Anaplasma* spp. *Ehrlichia* spp.-specific DNA was determined in duplicates. A Ct value ≤ 38 was considered as positive and values between 38 and 40 were considered as uncertain results as recommended by the supplier. Species identification was performed on qPCR positive and questionable samples using conventional PCR targeting the 16S rRNA gene and sequencing as described elsewhere [39].

2.4. Statistical Analysis

Data analysis were performed with SPSS Statistics software® (IBM Corp, Armonk, NY, USA, version 19). Seroprevalence is the proportion of positive results measured in serum within a population. Confidence interval (CI) was computed from binominal distribution of the obtained data (positivity in population). Odds ratios (ORs) were calculated using a relative risk option. In the present survey, possible risk factors such as age, tick infestation, animal husbandry system, age group (≤4 and >4 years), and keeping condition (stable/stationary, nomadic and posture) were analyzed. The Chi-square test was used to determine the association among categorized risk groups [40]. The multivariable regression model was used to evaluate the effect of multiple variables in the same model using ANOVA and F test for cELISA and real time PCR results.

2.5. Ethical Statement

This study was carried out in strict accordance with the recommendations of the Egyptian Network of Research Ethics Committees (ENREC), which complies with the international laws and regulations regarding ethical considerations in research. The ENREC approved this research work. For purposes of this study, all animal owners consented to sampling.

3. Results

Out of 758 tested serum samples, 140 were seropositive by cELISA, and the overall estimated seroprevalence of anaplasmosis was 18.5% (CI: 15.8–21.4%). In 61 investigated farms, 31 (50.8%) farms had seropositive cattle for anaplasmosis (Table 2.2). The results of seroprevalence for each governorates are shown in Table 2.2. The majority of seropositive animals were located in Gharbia (100%), Suez (83.3%) and Port Said (33.3%), while the lowest prevalence was recorded in Sohag (4.7%) and Aswan (5.2%). *Anaplasma*/*Ehrlichia*-specific DNA was detected in 5.3% (CI: 3.8–7.1%) of the seropositive samples by real time PCR. Species differentiation was attempted by 16S rRNA amplification and sequencing. Only four of all qPCR positive and questionable samples were positive for the 16S rRNA gene and showed 100% sequence identity with *A. marginale* (data not shown). Most of the PCR-positive animals were from the Nile Valley and Delta region (7.1%). Only 3.95% (30/758) of sera were serologically positive for *Coxiella burnetii* and *Anaplasma* spp. (Table 2.2).

Table 2.2. Prevalence of bovine anaplasmosis in 24 investigated governorates.

Domain	Governorate	No. of Animals tested	No. of Farms (Positive)	Prevalence No. (%)		Co-detection of *Coxiella* and *Anaplasma*
				cELISA	PCR	
Western Area	Matrouh	167	4 (4)	25 (15)	7 (4.2)	3
	New valley	170	6 (5)	36 (21.6)	9 (5.3)	8
Eastern Area	Red Sea	138	4 (3)	25 (18.5)	4 (2.9)	10
Nile Valley and Delta Area	Alexandria	9	3 (1)	1 (11.1)	0	0
	Assiut	33	2 (2)	10 (30.3)	2 (6.1)	2
	Aswan	58	3 (1)	3 (5.2)	2 (3.4)	2
	Cairo	12	2 (1)	2 (16.7)	0	0
	Dakahlia	11	2 (1)	2 (18.2)	1 (9.1)	0
	Damietta	12	2 (2)	3 (25)	3 (25)	0
	Fayoum	9	3 (2)	2 (22.2)	0	0
	Gharbia	2	1 (1)	2 (100)	2 (100)	0
	Ismailia	7	4 (2)	2 (28.5)	0	0
	Minya	12	2 (1)	1 (8.3)	1 (8.3)	0
	Port Said	12	2 (2)	4 (33.3)	3 (25)	0
	Qena	22	3 (2)	11 (50)	3 (13.6)	0
	Sohag	21	2 (1)	1 (4.8)	0	1
	Suez	12	2 (2)	10 (83.3)	3 (25)	4
	Beheira	1	1 (0)	0	0	0
	Beni-Suef	22	2 (0)	0	0	0
	Giza	9	3 (0)	0	0	0
	Kafr El Sheikh	7	3 (0)	0	0	0
	Menoufia	9	3 (0)	0	0	0
	Qualyubia	1	1 (0)	0	0	0
	Sharkia	2	1 (0)	0	0	0
Total		758	61 (33%)	140 (18.5%)	40 (5.3%)	30 (4%)

The number of positive samples per domain ranged between 18.1–19.08% and 2.9–7.1%, respectively (Table 2.3).

Sixty percent of cELISA-positive animals were older than 4 years, with 56.42% of those animals kept in stables/stationary and 36.42% being nomadic. Sixty-five percent of positive animals were infested with ticks (Table 2.3). Tick infestation was the only risk factor that had a significant association with bovine anaplasmosis ($\chi^2 = 9.36$, $p = 0.009$), which is reflected by an Odds ratio of 1.7. Detailed information about this risk factor analyses is displayed in Table 2.3. The multivariable regression model demonstrated no relationship between risk factors of anaplasmosis (cELISA < ANOVA; F (6,744) = 0.799, $p = 0.571$> / real time PCR < ANOVA; F (6,744) = 2.005, $p = 0.063$>).

Table 2.3. Potential risk-associated factors for bovine anaplasmosis in Egypt.

Risk Factor		c ELISA							Real Time PCR			
		No. of Positive Animals (No. of Suspicious Samples)			Seropositive	Odds Ratio	95% Confidence Interval (CI) Pos. (Pos. plus Suspicious)	Chi Square (df) (*p*-Value)	No. of Positive Animals (Suspicious)	DNA Positive Samples	95% Confidence Interval (CI)	Chi Square (df) (*p*-Value)
			Proportion in Positive Animals (Suspicious)	Proportion in Total Animals (Suspicious)								
Domain	Western Domain	61 (22)	43.57% (55%)	61/337 = 18.10% (6.52%)	18.10%	1.09	14.1–22.6%	$\chi^2(4) = 2.23$; $p = 0.69$	16 (9)	4.74%	2.7–7.6%	$\chi^2(6) = 9.01$; $p = 0.17$
	Nile Delta	54 (11)	38.57% (27.5%)	54/283 = 19.08% (3.88%)	19.08%	0.92	14.7–24.2%		20 (1)	7.1%	4.4–10.7%	
	Eastern Domain	25 (7)	17.85 (17.5%)	25/138 = 18.11% (5.07%)	18.11%	0.99	12.1–25.6%		4 (3)	2.9%	0.8–7.3%	
	Total	140 (40)		140/758 = 18.46% (5.27%)	18.5%	ND	15.8–21.4%		40 (13)	5.3%	3.8–7.1%	
Animal age group	≤4 years	56 (17)	40% (42.5%)	18.54% (5.62%)	18.54%	1.02	14.3–23.4%	$\chi^2(2) = 0.144$; $p = 0.93$	19 (7)	6.3%	3.8–9.7%	$\chi^2(3) = 2.57$; $p = 0.46$
	>4 years	84 (23)	60% (57.5%)	18.42% (5.04%)	18.42%	0.98	15.0–22.3%		21 (6)	4.60%	2.9–7%	
Animal husbandry	Stable/Stationary	79 (18)	56.42% (45%)	19.08% (4.34%)	19.1%	0.96	15.4–23.2%	$\chi^2(6) = 8.30$; $p = 0.21$	24 (4)	5.8%	3.7–8.5%	$\chi^2(9) = 8.82$; $p = 0.69$
	Nomadic	51 (22)	36.42% (55%)	16.45% (7.09%)	16.5%	0.98	12.5–21.1%		13 (8)	4.2%	2.3–7.1%	
	Nomadic & Pasture	10	7.14%	29.41%	29.41%	1.34	15.1–47.5%		3 (1)	8.8%	1.9–23.7%	
Tick infestation		91 (27)	65% (67.5%)	21.51% (6.38%)	19.45%	1.71	17.7–25.7%	$\chi^2(2) = 9.36$; $p = 0.009$[a]	26 (11)	6.1%	4.1–8.9%	$\chi^2(3) = 11.74$; $p = 0.45$
Animals kept separate		79 (18)	56.42% (45%)	19.45% (4.43%)	19.5%	1.02	15.7–23.6%	$\chi^2(2) = 1.64$; $p = 0.44$	24 (4)	5.9%	3.8–8.7%	$\chi^2(4) = 3.38$; $p = 0.33$
Another animal species living on farm		6 (1)	25% (4%)	24% (4%)	24%	ND	9.4–45.1%	ND	1 (2)	4%	0.1–20.4%	ND

Chi-square analysis calculated by ignoring the missing samples to avoid a high percentage of expected frequency below 5.[a] Demonstrated significant association for tick infestation. Both assays were conducted in duplicate. 'suspicious' means that samples have only one positive result.

4. Discussion

The aim of this study was to assess the prevalence of bovine anaplasmosis in Egypt to predict risk factors and provide baseline data for an effective design of disease control. Anaplasmosis has been recorded in cattle in Egypt for more than 50 years since it was first mentioned in 1966 [26], and was present in at least 22 of 27 governorates and the majority of positive samples reported from Suez, Dakahilia, Sharkir, Kafar el-Sheikh, Garbia, Manofia, and Minya [26]. Despite the evidence for endemicity of *Anaplasma* spp. in Egypt in official reports, a lack of data in the scientific literature is obvious. Only seven articles were found that provide data on anaplasmosis in cattle and one each in water buffaloes and camels. It is possible that the infections are more prevalent as reported, due to misdiagnosis and undetected carrier animals. It is not obvious why anaplasmosis does not get the expected attention from non-governmental scientists. This shows a strong need for more detailed information on the distribution of anaplasmosis in Egypt.

To understand the epidemiology of bovine anaplasmosis in Egypt, screening of sera collected for a previous Q fever survey were used to determine prevalence, risk factors and distribution of bovine anaplasmosis in Egypt.

In this study, the seroprevalence of anaplasmosis in Qena governorate was 50%, which is higher than that reported previously by Fereig et al. (28%) using a cELISA test [33]. Molecular investigation done by El-Ashkar et al. (2015) showed a high difference for the presence of *A. marginale*-specific DNA in sera when compared to the obtained data in this study, 20.12% vs 9.09%, respectively [27]. These discrepancies may be caused by different sampling times, sampling strategies and locations. It has to be noted that the samples in this study were taken on an independent, statistically-based sampling plan in contrast to sampling during locally limited outbreaks or samples taken from clinical practice. Most reports on bovine anaplasmosis were from animals, which were clinically ill or had a history of anaplasmosis. Screening by IFA was performed twice previously [35,36]. This test has several drawbacks i.e. limitations on the number of tests per day to be done by one operator and nonspecific fluorescence [6]. Hence, it is not recommended by OIE [6]. Studies using IFA for diagnosis of anaplasmosis cannot be compared to other studies using different OIE suitable assays i.e. cELISA. Six studies have used microscopic examination to confirm the agents near the margin of the erythrocyte. This method is recommended by the OIE for the confirmation of clinical cases of anaplasmosis. However, microscopy is not appropriate for prevalence studies and does not allow species differentiation [6]. The combination of cELISA and real time PCR proved to be easy in implementation in the laboratory and allows high throughput analysis of samples.

Chi square analyses resulted in a significant association for tick infestation with $\chi(2) = 9.36$ and $p = 0.009$. This finding was expected, as ticks are vectors of anaplasmosis. There is no significant association between anaplasmosis and Q fever ($\chi(6) = 6.27$, $p = 0.18$). In addition, the multivariable regression model indicated no dependency between risk factors and their relevance for anaplasmosis (cELISA < ANOVA; $F(6,744) = 0.799$, $p = 0.571$> / real time PCR < ANOVA; $F(6,744) = 2.005$, $p = 0.063$>).

Summarized data from Egyptian literature [27,33,34,36], official reports, and this work show that bovine anaplasmosis is present in the governorates Matrouh, Damietta, Dakahila, and Qena (except Qalybia due to in-availability of samples). An inconsistency of national reports and our results for Sharkia and Beheira are based on limited availability of samples. No official reports from Aswan and Red sea were available, but in the presented study, 5.17% and 18.51% were positive by cELISA, and thereof, 3.44% and 2.89% were PCR-positive for *Anaplasma* spp./*Ehlichia* spp.-specific DNA, respectively. Species differentiation using conventional PCR targeting the 16S rRNA gene was not successful. Only four samples yielded PCR products with 100% sequence identity to *A. marginale*. This might be due to a higher sensitivity of the qPCR assay compared to conventional PCR and a low amount and quality of DNA. Beni-Suef is the only governorate in which no outbreaks have been reported and was also found to have no positive samples in this study. Bovine anaplasmosis is present in neighboring governorates, but why it is absent from this governorate is unknown. We found antibodies specific for bovine anaplasmosis in 17 governorates, which coincides well with official statistics. The country has an enormous burden of diseases and outbreaks; effective control of bovine anaplasmosis should include

control of the tick vectors. In all domains, cattle were infested with ticks, which may be due to the unavailability of acaricides or access to information affecting the ability of animal owners to control ticks. This might also indicate that there is not sufficient veterinary care. Tick vaccines have a negative influence on tick feeding and reproduction [22] but are not available for field use yet. *Anaplasma centrale* live vaccines can give partial protection against bovine anaplasmosis and might be useful in future control programs. The role of nomadic husbandry in the dissemination of anaplasmosis is unknown and is still not investigated yet. The spread of diseases through human behavior [41], humans activities [42], and human mobility [43] are well known. The movement of carrier animals that do not display any obvious symptoms of anaplasmosis may be an additional factor to be considered.

5. Conclusion

National reports show that bovine anaplasmosis is widely distributed in Egypt. The results of this study confirm the nationwide and significant prevalence of bovine anaplasmosis. In order to reduce the spread of infection, more attention to control measures is required. Raising of awareness in decision makers of the public health and private sectors, especially veterinarians and animal owners, is an effective but simple way to improve the situation of anaplasmosis in a reasonably short time.

Author Contributions: Data curation, O.P.; Formal analysis, O.P.; Investigation, O.P. and K.M.-S.; Methodology, O.P. and K.M.-S.; Supervision, H.E.-A., F.M., U.R., H.N. and K.M.-S.; Writing—original draft, O.P. and H.N.; Writing—review & editing, O.P., H.E.-A., U.R., H.N. and K.M.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by German Federal Foreign Office, Germany grant number [AA-OR-12-370.43 BIOS FLI EGY] and the APC was funded by Institute of Bacterial Infections and Zoonoses, Friedrich-Loeffler-Institut, Jena, Germany.

Acknowledgments: The authors thank German Federal Foreign Office, Germany "German Biosecurity program" in collaboration with Friedrich-Loeffler-Institut, Institute of Bacterial Infections and Zoonoses, Jena, Germany, for their support to carry out this work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Theiler, A. Gall-sickness of South Africa. (Anaplasmosis of Cattle.). *J. Comp. Pathol. Therap.* **1910**, *23*, 98–115, doi:10.1016/S0368-1742(10)80028-1.
2. Ueti, M.W.; Reagan, J.O.J.; Knowles, D.P.J.; Scoles, G.A.; Shkap, V.; Palmer, G.H. Identification of midgut and salivary glands as specific and distinct barriers to efficient tick-borne transmission of *Anaplasma marginale*. *Infect. Immun.* **2007**, *75*, 2959–2964, doi:10.1128/IAI.00284-07.
3. Rikihisa, Y. Mechanisms of obligatory intracellular infection with *Anaplasma phagocytophilum*. *Clin. Microbiol. Rev.* **2011**, *24*, 469–489, doi:10.1128/CMR.00064-10.
4. Merck. The Veterinary Manual: Anaplasmosis. Availabe online: https://www.msdvetmanual.com/circulatory-system/blood-parasites/anaplasmosis (accessed on 31 July 2019).
5. Kocan, K.M.; de la Fuente, J.; Blouin, E.F.; Coetzee, J.F.; Ewing, S.A. The natural history of *Anaplasma marginale*. *Vet. Parasitol.* **2010**, *167*, 95–107, doi:10.1016/j.vetpar.2009.09.012.
6. OIE. Bovine Anaplasmosis. Availabe online: http://www.oie.int/standard-setting/terrestrial-manual/access-online/ (accessed on 17 June 2019).
7. Aubry, P.; Geale, D.W. A review of bovine anaplasmosis. *Transbound. Emerg. Dis.* **2011**, *58*, 1–30, doi:10.1111/j.1865-1682.2010.01173.x.
8. Constable, P.D.; Hinchcliff, K.W.; Done, S.H.; Grünberg, W. Diseases of the Hemolymphatic and Immune Systems. In *Veterinary Medicine*, 11th ed.; Saunders, W.B., Ed.; Saunders Ltd.: London , UK, 2017; doi:10.1016/B978-0-7020-5246-0.00011-5.
9. Ioannou, I.; Chochlakis, D.; Kasinis, N.; Anayiotos, P.; Lyssandrou, A.; Papadopoulos, B.; Tselentis, Y.; Psaroulaki, A. Carriage of *Rickettsia* spp., *Coxiella burnetii* and *Anaplasma* spp. by endemic and

migratory wild birds and their ectoparasites in Cyprus. *Clin. Microbiol. Infect.* **2009**, *15* (Suppl. 2), 158–160, doi:10.1111/j.1469-0691.2008.02207.x.
10. Mărcuţan, I.D.; Sándor, A.D.; Mihalca, A.D.; Gherman, C.M.; Kalmár, Z.; D'Amico, G.; Dumitrache, M.O.; Cozma, V. Prevalence of *Anaplasma phagocytophilum* in ticks collected from migratory birds in Danube Delta, Romania. *Parasites Vectors* **2014**, *7*, P16–P16, doi:10.1186/1756-3305-7-S1-P16.
11. Ybañez, A.P.; Inokuma, H. *Anaplasma* species of veterinary importance in Japan. *Vet. World* **2016**, *9*, 1190–1196, doi:10.14202/vetworld.2016.1190-1196.
12. Henniger, T.; Henniger, P.; Grossmann, T.; Distl, O.; Ganter, M.; von Loewenich, F.D. Congenital infection with *Anaplasma phagocytophilum* in a calf in northern Germany. *Acta Vet. Scand.* **2013**, *55*, 38, doi:10.1186/1751-0147-55-38.
13. Silaghi, C.; Santos, A.S.; Gomes, J.; Christova, I.; Matei, I.A.; Walder, G.; Domingos, A.; Bell-Sakyi, L.; Sprong, H.; von Loewenich, F.D.; et al. Guidelines for the Direct Detection of *Anaplasma* spp. in Diagnosis and Epidemiological Studies. *Vector Borne Zoonotic Dis.* **2017**, *17*, 12–22, doi:10.1089/vbz.2016.1960.
14. McDaniel, C.J.; Cardwell, D.M.; Moeller, R.B.J.; Gray, G.C. Humans and cattle: A review of bovine zoonoses. *Vector Borne Zoonotic Dis.* **2014**, *14*, 1–19, doi:10.1089/vbz.2012.1164.
15. Annen, K.; Friedman, K.; Eshoa, C.; Horowitz, M.; Gottschall, J.; Straus, T. Two cases of transfusion-transmitted *Anaplasma phagocytophilum*. *Am. J. Clin. Pathol.* **2012**, *137*, 562–565, doi:10.1309/ajcp4e4vqqqoziaq.
16. Jereb, M.; Pecaver, B.; Tomazic, J.; Muzlovic, I.; Avsic-Zupanc, T.; Premru-Srsen, T.; Levicnik-Stezinar, S.; Karner, P.; Strle, F. Severe Human Granulocytic Anaplasmosis Transmitted by Blood Transfusion. *Emerg. Infect. Dis.* **2012**, *18*, 1354–1357, doi:10.3201/eid1808.120180.
17. Matthew Waxman, M.D. *Anaplasma phagocytophilum* Transmitted Through Blood Transfusion-Minnesota 2007. *Ann. Emerg. Med.* **2009**, *53*, 643–645, doi:10.1016/j.annemergmed.2009.03.010.
18. Quinn, P.J. Rickettsiales and *Coxiella burnetii*. In *Concise Review of Veterinary Microbiology*, 2nd ed.; Wiley & Sons Ltd: Hoboken, NJ, USA, 2016.
19. Chung, C.; Wilson, C.; Bandaranayaka-Mudiyanselage, C.B.; Kang, E.; Adams, D.S.; Kappmeyer, L.S.; Knowles, D.P.; McElwain, T.F.; Evermann, J.F.; Ueti, M.W.; et al. Improved diagnostic performance of a commercial *Anaplasma* antibody competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5–glutathione S-transferase fusion protein as antigen. *J. Vet. Diagn. Investig.* **2013**, *26*, 61–71, doi:10.1177/1040638713511813.
20. Kocan, K.M.; Blouin, E.F.; Barbet, A.F. Anaplasmosis control. Past, present, and future. *Ann. N. Y. Acad. Sci.* **2000**, *916*, 501–509, doi:10.1111/j.1749-6632.2000.tb05329.x|.
21. Kopáček, P.; Hajdušek, O.; Burešová, V.; Daffre, S. Tick Innate Immunity. In *Invertebrate Immunity*; Söderhäll, K., Ed.; Springer: Boston, MA, USA, 2010; pp. 137–162, doi:10.1007/978-1-4419-8059-5_8.
22. Merino, O.; Alberdi, P.; Pérez de la Lastra, J.M.; de la Fuente, J. Tick vaccines and the control of tick-borne pathogens. *Front. Cell. Infect. Microbiol.* **2013**, *3*, 30–30, doi:10.3389/fcimb.2013.00030.
23. Hove, P.; Khumalo, Z.T.H.; Chaisi, M.E.; Oosthuizen, M.C.; Brayton, K.A.; Collins, N.E. Detection and Characterisation of *Anaplasma marginale* and *A. centrale* in South Africa. *Vet. Sci.* **2018**, *5*, doi:10.3390/vetsci5010026.
24. Okafor, C.C.; Collins, S.L.; Daniel, J.A.; Harvey, B.; Sun, X.; Coetzee, J.F.; Whitlock, B.K. Factors associated with Seroprevalence of *Anaplasma marginale* in Kentucky cattle. *Vet. Parasitol. Reg. Stud. Rep.* **2018**, *13*, 212–219, doi:10.1016/j.vprsr.2018.07.003.
25. Marcondes, C.B. Anaplasmosis. In *Arthropod Borne Diseases*; Springer International Publishing: Cham, Switzerland, 2017; doi:10.1007/978-3-319-13884-8.

26. CAPMAS. Animal Diseases. Availabe online: http://www.capmas.gov.eg/Pages/Publications.aspx?page_id=5104&Year=23381; http://www.t-series.capmas.gov.eg/agreculur.aspx; https://www.capmas.gov.eg/ (accessed on 17 June 2019).
27. El-Ashker, M.; Hotzel, H.; Gwida, M.; El-Beskawy, M.; Silaghi, C.; Tomaso, H. Molecular biological identification of *Babesia*, *Theileria*, and *Anaplasma* species in cattle in Egypt using PCR assays, gene sequence analysis and a novel DNA microarray. *Vet. Parasitol.* **2015**, *207*, 329–334, doi:10.1016/j.vetpar.2014.12.025.
28. El-Ashker, M.; Salama, M.; El-Sebaei, M.; Risha, E.; Abdelhamid, F.; El-Diasty, M.; El-Fadle, E. Significance of clinical variables and selected biochemical markers in predicting the outcome of bovine anaplasmosis. *Veterinární Med.* **2016**, *60*, 301–308, doi:10.17221/8244-vetmed.
29. Radwan, M.E.I.; Ali, A.; Abd elhamied, O. Epidemiological Studies ,Molecular Diagnosis of *Anaplasma marginale* in Cattle and Biochemical Changes Associated with it in Kaliobia Governorate. *Am. J. Infect. Dis. Microbiol.* **2013**, *1*, 46–49, doi:10.12691/ajidm-1-3-2.
30. Abdel Hamid, O.M.; Radwan, M.E.I.; Ali, A. Biochemical changes associated with *Anaplasma* infection in cattle. *Glob. J. Biotechnol. Biochem.* **2014**, *9*, 19–23, doi:10.5829/idosi.gjbb.2014.9.1.8290.
31. Elhariri, M.D.; Elhelw, R.A.; Hamza, D.A.; Soliman, D.E. Molecular detection of *Anaplasma marginale* in the Egyptian water bufaloes (*Bubuloes bubalis*) based on major surface protein 1α. *J. Egyp. Soc. Parasitol.* **2017**, *47*, 247–252.
32. El-Naga, T.R.; Barghash, S.M. Blood Parasites in Camels (*Camelus dromedarius*) in Northern West Coast of Egypt. *J. Bacteriol. Parasitol.* **2016**, *7*, doi:10.4172/2155–9597.1000258.
33. Fereig, R.M.; Mohamed, S.G.A.; Mahmoud, H.; AbouLaila, M.R.; Guswanto, A.; Nguyen, T.T.; Ahmed Mohamed, A.E.; Inoue, N.; Igarashi, I.; Nishikawa, Y. Seroprevalence of *Babesia bovis*, *B. bigemina*, *Trypanosoma evansi*, and *Anaplasma marginale* antibodies in cattle in southern Egypt. *Ticks Tick Borne Dis.* **2017**, *8*, 125–131, doi:10.1016/j.ttbdis.2016.10.008.
34. El-Naga, T.R.; Mahmoud., M.A.; Osman., W.A.; Goda, A.S.A. Serological survey of *Anaplasma marginale* (*Rickettsia*) antibodies in animal by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. *Suez Canal Vet. Med. J. (SCVMJ)* **2009**, 309–319.
35. Younis, E.E.; Hegazy, N.A.M.; El-Deeb, W.; El-Khatib, R.M. Epidemiological and biochemical studies on bovine anaplamosis in dakahlia and demiatta governorates in Egypt. *Bull. Anim. Health Prod. Afr.* **2009**, *57*, doi:10.4314/bahpa.v57i4.51668.
36. Salm, F.F.; Younis, E.E.; Hegazy, N.M.; El-Sawalhy, A.A. Epidemiological studies on bovine anaplasmosis. In *Bulletin of Animal Health and Production in Africa*; Inter-African Bureau for Animal Resources: Nairobi, Kenya, 2011; pp. 179–189.
37. Loftis, A.D.; Reeves, W.K.; Szumlas, D.E.; Abbassy, M.M.; Helmy, I.M.; Moriarity, J.R.; Dasch, G.A. Rickettsial agents in Egyptian ticks collected from domestic animals. *Exp. Appl. Acarol.* **2006**, *40*, 67–81, doi:10.1007/s10493-006-9025-2.
38. VMRD. Product Catalog. Availabe online: https://www.vmrd.com/; https://www.vmrd.com/core/files/vmrd/uploads/files/VMRD%20Catalog_4_2_18.pdf (accessed on 17 July 2019).
39. Stuen, S.; Nevland, S.; Moum, T. Fatal cases of Tick-borne fever (TBF) in sheep caused by several 16S rRNA gene variants of *Anaplasma phagocytophilum*. *Ann. N. Y. Acad. Sci.* **2003**, *990*, 433–434, doi:10.1111/j.1749-6632.2003.tb07407.x.
40. Kim, H.Y. Statistical notes for clinical researchers: Chi-squared test and Fisher's exact test. *Restor. Dent. Endod.* **2017**, *42*, 152–155, doi:10.5395/rde.2017.42.2.152.
41. Funk, S.; Salathé, M.; Jansen, V.A.A. Modelling the influence of human behaviour on the spread of infectious diseases: A review. *J. R. Soc. Interface* **2010**, *7*, 1247–1256, doi:10.1098/rsif.2010.0142.
42. Lindahl, J.F.; Grace, D. The consequences of human actions on risks for infectious diseases: A review. *Infect. Ecol. Epidemiol.* **2015**, *5*, 30048–30048, doi:10.3402/iee.v5.30048.

43. Meloni, S.; Perra, N.; Arenas, A.; Gómez, S.; Moreno, Y.; Vespignani, A. Modeling human mobility responses to the large-scale spreading of infectious diseases. *Sci. Rep.* **2011**, *1*, 62, doi:10.1038/srep00062.

C H A P T E R 3

Performance Analysis of Anaplasma Antibody Competitive ELISA Using the ROC Curve for Screening of Anaplasmosis in Camel Populations in Egypt

Pathogens. 2020; 9(3), 64; Published: 27 February 2020
doi: 10.3390/pathogens9030165

pathogens

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Performance Analysis of *Anaplasma* Antibody Competitive ELISA Using the ROC Curve for Screening of Anaplasmosis in Camel Populations in Egypt

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Received: 21 January 2020; Accepted: 25 February 2020; Published: date

Abstract: Anaplasmosis is a tick-born and potential zoonotic disease caused by *Anaplasma* (*A.*) *phagocytophilum*, *A. ovis*, *A. platys* and *A. capra*. *Anaplasma marginale* affecting bovines and camels causing significant economic losses. Camels as an integral part of the socio-economic lifestyle of nomads in semi-arid to arid ecosystems are prone to suffer from subclinical *Anaplasma* infections. This study aimed to determine the performance and adaptation of commercial competitive *Anaplasma* ELISA (cELISA) as a tool for screening the seroprevalence of anaplasmosis whitin the camel populations in Egypt. This study was based on the serological investigation of 437 camel sera collected between 2015 and 2016 during a Q fever prevalence study in Egypt using commercially available cELISA for the detection of antibodies specific for *Anaplasma* in bovine serum. The receiver operating characteristic (ROC) curve, an analysis method for optimizing cutoff values in cELISAs, was used to estimate the sensitivity and specificity using 76 true as serological positive ($n = 7$) and negative ($n = 60$) for *Anaplasma* antibodies. ROC curve analysis was done for 7 true positive and 60 true negative bovine samples and 7 true positive and 29 true negative camel samples serum. Real time PCR and/or conventional PCR was applied to confirm *Anaplasma* spp. specific-DNA in camel serum as an indication of a true positive and true negative for ROC analysis. Chi square analysis was performed to estimate the association between risk factors and anaplasmosis in camels. The cutoff value was determined as 0.42 (p value= < 0.001). Data simulation with randomly generated values revealed a cutoff value of 0.417 (p = < 0.001) with resulting 58.1% Se and 97.8% Sp. Seven true positive and 29 true negative camel serum samples was confirmed by PCR. Using the estimated cut off, the seroprevalence in the Nile Valley and Delta and the Eastern Desert domain was 47.4% and 46.4%, respectively. The potential risk factors as domains and origin of animals were less significantly associated with the prevalence of anaplasmosis (domains: $\chi(2) = 41.8$, p value ≤ 0.001 and origin: $\chi(2) = 42.56$, p value = < 0.001). Raising awareness especially for veterinarians and animal owners will significantly contribute to the best understanding of anaplasmosis in camels in Egypt. Alternative (*in silico*) validation techniques and preliminary prevalence studies are mandatory towards the control of neglected anaplasmosis in the camel population.

Keywords: anaplasmosis; camel; ROC curve; real time PCR; cELISA

1. Introduction

Camels are utilized for milk, meat, wool and hide production as well as for transport since 4000 BC [1]. Most camel populations are kept in India and at the Horn of Africa [1]. In Egypt, the camel population has steadily increased between 2002 and 2015 [2].

Anaplasma and *Ehrlichia* are obligate intracellular alphaproteobacteria and belonging to order Rickettsiales, family Anaplasmataceae that are transmitted to vertebrate hosts by ticks of the family Ixodidae and cause symptoms similar to febrile diseases in humans and domestic animals like the camel [3,4]. Anaplasmosis often occurs in animals of tropical and subtropical regions but also in North America, Europe and the Mediterranean region [3,5]. Anaplasmosis can be transmitted mechanically by ticks, tabanid vectors, iatrogenically and transplacentally [5]. Anaplasmosis usually manifests as a subclinical infection or as co-infection in camels [6]. El-Naga and Barghash, 2016 reported clinical cases with fever, enlarged lymph nodes, anemia and jaundice in camels [7]. Other studies and deposited sequences (NCBI) indicated the presence of *Anaplasma camelii*, *A. marginale*, *A. centrale*, *A. ovis* and *A. platy* DNA in camels [8].

Routine diagnosis of anaplasmosis in camels is based on clinical signs and microscopic examination of blood samples. Proper selection of currently available diagnostic assays to obtain the maximal confirmation potential was dependent upon recording the detailed clinical history that identifies the time interval from the onset of symptoms appearance to the investigation of the clinical specimens [9].

Although the indirect fluorescent antibody technique (IFAT) is one of the most commonly used tests, ELISA has more advantages over it, since results can be obtained directly through a microplate reader, which make it possible to evaluate a larger number of serum samples and avoiding problems with doubtful interpretations [10].

Real-time PCR assay is considered as a rapid, sensitive and accurate diagnostic adjunct when compared with direct blood smear analysis for the identification of anaplasmosis. Serologic detection correlates poorly with PCR or blood smear analysis and more accurately reflects the collective exposure history occurring from late in the acute infection period into convalescence [9].

Statistical approaches can significantly help amending the performance of analytical tests. Receiver operating characteristic (ROC) curve analysis [11] and a World Organisation for Animal Health (OIE) recommended tool [12] were commonly used to optimize the cutoff values in ELISAs to find the best correlation for sensitivity (*Se*) and specificity (*Sp*) [13–16]. Some other methods to estimate the cutoff values are (1) mean value plus three standard deviations of negative controls [17]; (2) $Cutoff = \overline{X}\text{neg} + 0.13\ \overline{X}\text{pos}$ where $\overline{X}$ is the mean [18,19] and (3) $Cutoff = \overline{X} + fSD''$ with $f = t\sqrt{1 + (1/n)}$ [19,20]. These methods are based on values obtained with negative sera. Frey et al. (1998) relied on the upper tail of the *t*-distribution of negative samples [20].

Anaplasmosis has been reported in some parts of Egypt in cattle, buffaloes, camels and humans. Nevertheless, there is a lack of regular monitoring and countermeasure programs in the field. *Anaplasma marginale* is most often reported and confirmed in cattle, camels and arthropods from various host animal species. Anaplasmosis in camels was reported in Matrouh, South Sinai, Assuit and Luxor in Egypt. The diagnosis of anaplasmosis in Egypt was dependent on cELISA, IFA, microscopic examination and PCR [7,21–29].

A comprehensive prevalence study of camel anaplasmosis in Egypt and the adaptation of the commercial cELISA used for bovine to test camel sera are missing. Thus, this study aimed to adapt the commercial competitive ELISA (cELISA) used in bovines for camel sera and preliminary camel sera prevalence was analyzed.

2. Materials and Methods

2.1. Sampling and Serological Testing

Serum samples used in this study were originally collected between October 2015 and March 2016 in Egypt for a Q fever screening study in Egypt [30].

In total, 437 camel sera were collected from 24 governorates in Egypt. There were no sample collected from Sinai, Assuit, and Minya. Governorates were assigned into three domains: the *Western Desert*, the *Eastern Desert* the *Nile Valley* and the *Delta* region (Figure 3.1).

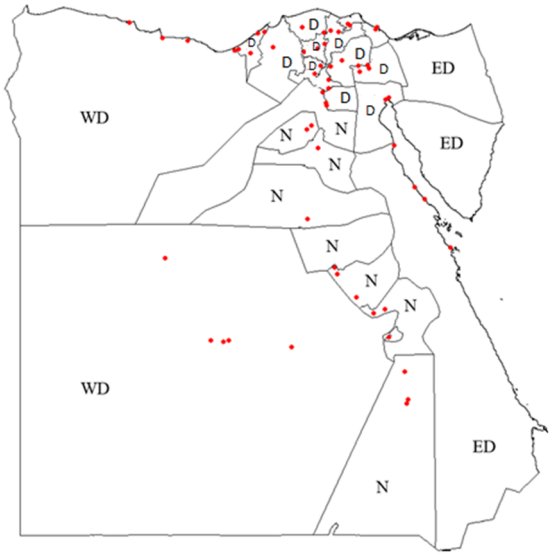

Figure 3.1. Geographical location of randomly selected sampling sites (red dots) in Egypt using GPS data Delta (D), Nile Valley (N), Western Desert (WD) and Eastern Desert (ED).

Data including age (≤4 or >4 years), husbandry system (stable/stationary, pasture and nomadic) and tick infestation were recorded in Table 3.1.

Table 3.1. Number (%) of animals sampled per domain with age group, origin of animals, husbandry systems and number of camel infested with ticks.

Domain		**Western Desert 193 (44.2%)**	**Nile Valley and Delta 175 (40%)**	**Eastern Desert 69 (15.8%)**	**Total Samples 437**
Age	≤4 years	32 (16.6%)	48 (27.4%)	17 (24.6%)	97 (22.2%)
	>4 years	161 (83.4%)	127 (72.6%)	52 (75.4%)	340 (77.8%)
Origin (Egypt/other country)		193/0 (100%/0)	13/162 (7.4%, 92.6%)	0/69 (0/100%)	206/231 (47.1%/52.9%)
Husbandry	Stable	0	15 (8.6%)	0	15 (3.4%)
	Nomadic	193 (100%)	133 (76.0%)	69 (100%)	395 (90.4%)
	Missing	0	27 (15.4%)	0	27 (6.2%)
Tick infestation		0	13 (7.4%)	21 (10.0%)	34 (7.78%)

Sera were screened for specific antibodies against *Anaplasma* spp. using a commercial competitive ELISA v2 (Veterinary Medical Research and Development Inc., Pullman, WA, USA) for the detection of antibodies specific for *Anaplasma* in bovine serum samples according to the manufacturer's instruction. This assay had a sensitivity (98%) and specificity of 100% in bovines, which were calculated from data generated by diagnostic laboratory field testing [31].

Additionally, 67 cattle samples, previously tested as serological positive (n = 7) and negative (n = 60) for *Anaplasma* antibodies were included as positive and negative control serum. ROC was used to evaluate the prediction of sensitivity and specificity [32].

2.2. DNA Preparation and PCR Amplification

DNA was extracted from seropositive and seronegative serum samples using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. The concentration and quality analysis of DNA in each sample was measured using a Nano-drop1000®

(Thermo Fisher, Wilmington, NC, USA). DNA amplification was done using real time- and/or conventional PCR.

The real time TaqMan™ PCR was performed using the AmpliTest *Anaplasma/Ehrlichia* spp. Kit (Amplicon Ltd., Wrocław, Poland) for quantitative detection of *Anaplasma* DNA according to the manufacturer's guidelines. The result of the cycle threshold (Ct) value ≤ 38 was considered 'positive' and samples had a Ct value between 38 and 40 were considered 'suspected'.

Conventional PCR was performed as described previously [32]. The PCR reaction was done using a Phusion Flash High-Fidelity PCR Master Mix (Thermo Fisher, Darmstadt, Germany) and primers MSP-5 254 F: 5'-GCA TAG CCT CCG CGT CTT TC-3' and MSP-5 779R: 5'-ACA CGA AAC TGT ACC ACT GCC-3' to amplify a 525 bp fragment of the major surface protein (MSP5) gene

2.3. Performed ROC Analyses

Diagnostic specificity, sensitivity and predictive values were determined by receiver operating characteristic (ROC) analysis (MedCalc statistical software, version 9.3.0.0). Based on the optical density (OD) values of the cELISA, positive and negative results ROC can be generated. Usually these data are good coverage, which means that all values are within the control range.

$$Control\ interval = \text{mean of Pos./Neg control} \pm 3 * standard\ deviation \quad (1)$$

$$\begin{aligned} &True\ pos.baseline \\ &= [mean\ of\ pos.control - 2 \\ &* standard\ deviation\ (smallest\ OD\ value), mean\ of\ pos.control + 2 \\ &* standard\ deviation\ (greatest\ OD\ value)] \end{aligned} \quad (2)$$

$$\begin{aligned} &True\ neg.baseline \\ &= [mean\ of\ neg.control - 2 \\ &* standard\ deviation\ (smallest\ OD\ value), mean\ of\ neg.control + 2 \\ &* standard\ deviation\ (greatest\ OD\ value)] \end{aligned} \quad (3)$$

A true positive and negative baseline established the probabilities of positivity or negativity were calculated to determine the upper/lower margin (limit) of the distribution of the control sera. The sera with the closest values to this limit can be selected as the true positive and negative range, due to the highest probability of positivity/negativity for further analyses.

ROC curve analysis was done for 7 true positive and 60 true negative bovine samples and 7 true positive and 29 true negative camel serum using SPSS Statistics software® (Armonk, IBM Corp, USA, version 19) to obtain Ct, Se and Sp values. These values were used to determine seroprevalence of 347 camel sera. In addition, the above formula was used for screened camel sera, baseline values were obtained true positive and true negative data for using in simulation analysis. In the simulation analysis of the 2300 field serum samples, random data (true negative = 2000 and true positive = 300) were generated using the positivity and negativity area of each plate.

ROC analysis for data reconstruction was done with 10% expected error. It should be noticed that wells with an optical density ≤ 0.20 were uncolored when inspected visually to assure a higher probability of positivity. In addition, for this study true positive/true negative samples were confirmed with real time PCR and/or conventional PCR with the exception of a true negative of bovine. These were selected from a true negative baseline.

2.4. Statistical Analyses

The metadata of collected serum in this study were categorized in age (≤4 and >4 years), tick infestation and the animals husbandry system (stable/nomadic). A chi-square or Fisher's exact test was used to determine the association of the disease with these risk factors. Seroprevalences were calculated as the proportion of positive results in a population.

3. Results

Seven true positive and 29 true negative camel serum samples were confirmed by real time PCR as an indication of the true positive and true negative for ROC analysis.

The results of statistical analyses for threshold optimization of the cELISA V2 for use in cattle (Figure 3.2A) and camel (Figure 3.2B) sera are shown in Table 3.2 and Figure 3.2. These values were 0.42 ($p = < 0.001$) in camels and 0.4022 ($p = < 0.001$) in cattle.

Table 3.2. Detailed data of receiver operating characteristic (ROC) analysis for cattle, camels and a simulation for camels.

Animal Species	Samples		Area Under the Curve					Coordinates of the Curve		
						Asymptotic 95% Confidence Intervals				
	Positive	Negative	Area	Std. Error	Asymptotic Signs	Low Bound	Upper Bound	Positive	Sensitivity	Specificity
Cattle	7	60	1.000	0.000 (<001)	0.000 (<001)	1	1	≤0.18	0.857	0 (100%)
								≤0.40 *	1 *	0 (100%) *
								≤0.61	1	0.017 (98.3%)
Camels	7	29	1.000	0.000 (<001)	0.000 (<001)	1	1	≤0.33	0.857	0 (100%)
								≤0.42 *	1 *	0 (100%) *
								≤0.51	1	0.034 (96.6%)
Simulation for camels	470	1830	0.779	0.015	0.000 (<001)	0.750	0.807	≤0.42	0.581	0.021 (97.7%)
								≤0.42 *	0.581 *	0.022 (97.8%) *
								≤0.42	0.581	0.022 (97.8%)

* Cut off vallues, *Se* and *Sp*.The simulation data were randomly generated after the true positive/true negative baseline for each plate was predicted based on the formula in the Materials and Methods.

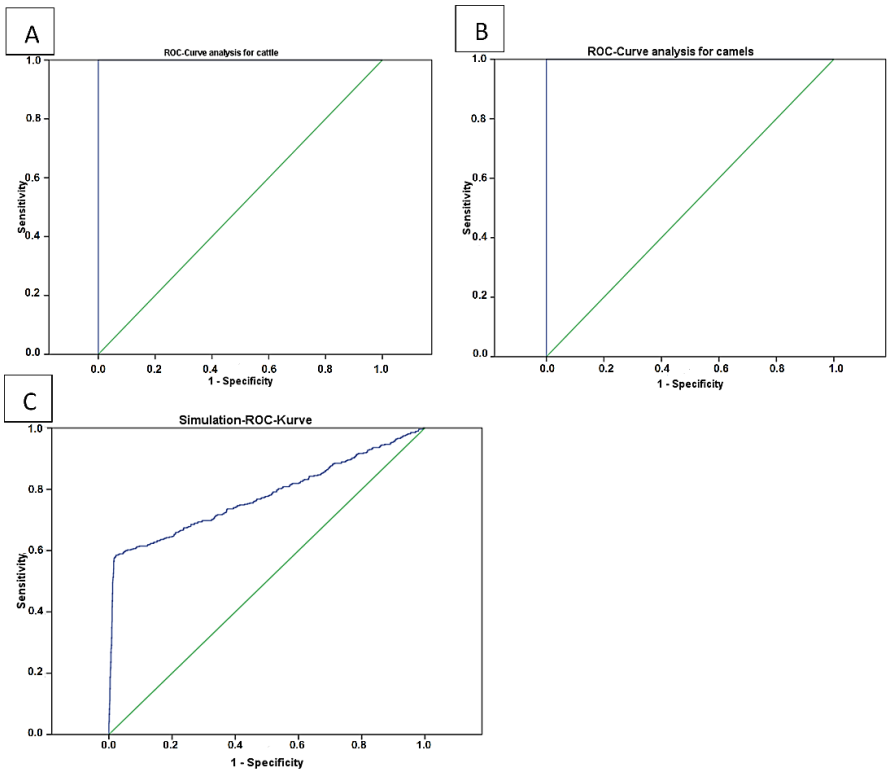

Figure 3.2. Display of the performance analysis of the cELISA Anaplasma kit V2 using true positive and true negative samples. Both analyses showed 100% *Se* and *Sp* (**A**: cattle and **B**: camels). A simulation (**C**) was done with 2300 randomly generated data involved positives (300) or negatives (2000). This data contain a 10% intentional error.

A scatter plot of the mean optical density from cattle sera values *vs.* the sera of camels showed a correlated relationship (Figure 3.3). Percent differences *vs*. mean results of cattle and camel sera provided average discrepancy reported error estimates and true errors, which shows the true extend of

the bias at a low optical density (Figure 3.3) [33,34]. This analysis proved good correlation between two tests in cattle and camel serum.

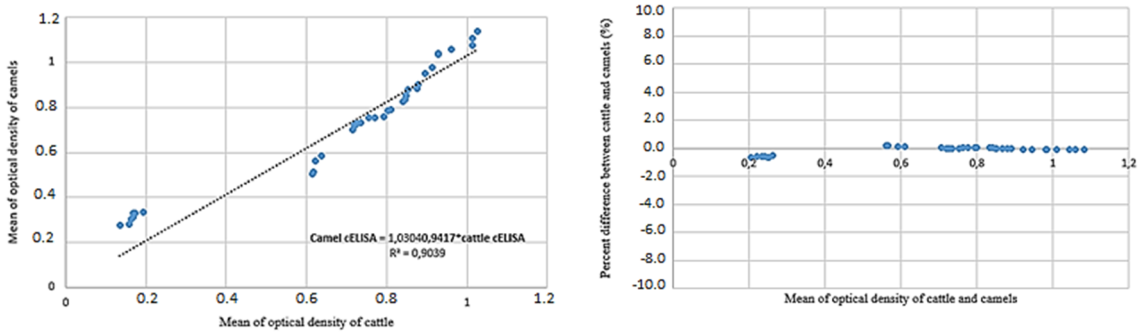

Figure 3.3. A scatter plot of values of the cELISA Anaplasma kit V2 in camel vs. cattle sera that shows good correlation between two tests. This good agreement favors the use in camels. The percent difference between the analysis of cattle and camel sera is showed the true extent of the bias of optical density (OD). This means that in this case the number of infected animals may be a little bit less/greater than in reality.

Data simulation with randomly generated values revealed a cutoff value of 0.417 (p = <0.001) with resulting 58.1% *Se* and 97.8% *Sp*.

The overall seroprevalence of anaplasmosis in camels (34.1%) was detected after optimization of the cELISA cutoff (Ct = 0.42). Nile Valley and Delta and Eastern Desert domains showed 47.4% and 46.4% seroprevalences, respectively. Of the camels 95.7% that were kept nomadic showed 33.7% seroprevalence.

There was no significant associated between anaplasmosis and age, the husbandry system and tick infestation (Table 3.3). The overall rate of camels infested with ticks was 10.7%. Camels younger than 4 years were highly infected than older (41.2% *vs.* 32.1%). Domain and origin of animals were found to be less significant associated risk factors for camel anaplasmosis (Table. 3.3).

Table 3.3. Associated risk factors for anaplasmosis in camels in Egypt.

Risk Factors		cELISA			Chi-Quadrat-Pearson	Phi and Cramer Value
		No. of Positive Animals				
		Proportion in Total Positive Animals (%)		Proportion in Population (Seroprevalence)		
Domain	Western Desert	34	22.8	17.6	X(2)=41.8 (p value= < 0.001)	0.309 (p value= < 0.001)
	Nile Valley and Delta	83	55.7	47.4		
	Eastern Desert	32	21.5	46.4		
	Total	149	100	34.1		
Origin (Egypt/other country)		39/110	26.2/72.5	18.9/48.6	X(2)=42.568 (p value= <0.001)	0.312 (p value = <0.001)
Age group	≤ 4 years	40	22.2	41.2	X(1)=2.899 (p value = 0.093)	0.080 (p value = 0.093)
	> 4 years	109	77.8	32.1		
Husbandry	Stable	6	4.3	0.4	X(1)=0.258 (p value = 0.61)	0.025 (p value = 0.611)
	Nomadic	133	95.7	33.7		
	missing	10	6.7	10/27=37		
Tick infestation		16	10.7	47.1	X(2)=3.819 (p value = 0.148)	0.0930 (p value = 0.148)

The majority of seropositivity 77.4% (n = 31) was determined in Aswan governorate from Nile Valley and Delta followed by 46.4% (n = 69) in red sea from Eastern Desert (Table 3.4).

Table 3.4. Seroprevalence of anaplasmosis in camels in different governorates using cELISA.

Domain	Governorate	No. of Tested Camels	Seroprevalence *n* (%)
Western Desert Area	Matrouh	91	12 (13.2%)
	New valley	102	22 (21.6%)
Eastern Desert Area	Red Sea	69	32 (46.4%)
Nile valley and Delta Area	Alexandria	8	1 (12.5%)
	Aswan	31	24 (77.4%)
	Beheira	8	2 (2.5.0%)
	Beni-Suef	10	5 (50.0%)
	Cairo	8	3 (37.5%)
	Dakahlia	8	3 (37.5%)
	Damietta	8	3 (37.5%)
	Fayoum	8	3 (37.5%)
	Gharbia	6	2 (33.3%)
	Giza	7	3 (42.9%)
	Ismailia	7	2 (28.6%)
	Kafr el-Sheikh	5	3 (60.0%)
	Luxor	9	6 (66.7%)
	Menofia	7	5 (71.4%)
	Port Said	8	3 (37.5%)
	Qena	11	4 (36.4%)
	Qualyubia	1	1 (100%)
	Sharkia	7	3 (42.9%)
	Sohag	10	5 (50.0%)
	Suez	8	2 (25.0%)
Total		437	149 (34.7%)

4. Discussion

Anaplasmosis is known in Egypt since 1966 in bovines and the presence of various species of *Anaplasma* were confirmed by the use of PCR in Egypt [7].

The descriptive and analytic epidemiological methods to describe the dynamics, prevalence and risk factors of infected populations through an improved process for data collection and plan for novel interventions helps to improve the understanding of the disease and its control [35,36].

The commercial *Anaplasma* cELISA V2 kit from Pullman, USA, has been previously validated for use in the diagnosis of *A. ovis* in sheep with 100% specificity (95% CI: 96.7%–100%) and 100% sensitivity (95% CI: 95.7%–100%) [15] and with 96.5% sensitivity and 98.1% specificity [16].

No commercial serological test available for the detection of anti-*Anaplasma* antibodies in camel serum. Thus, there was a clear need for first steps to adopt a bovine test kit for use in camels. This study was aimed to validate the commercially available cELISA for screening the anaplasmosis in camel serum. Subsequently this optimization test was used to estimate a preliminary prevalence of anaplasmosis in the Egyptian camel population.

Due to a lack of a sufficient pool of true negative and true positive sera, an in silico simulation for 2300 randomly generated data with 10% error has been done and resulted in 97.8% *Sp*. and 58.1% *Se*. The calculated lower sensitivity of the test in this study may have resulted from the included error for estimating the true positive and true negative range. In some test plates, few camel sera had a higher optical density than the optical density of the negative controls. This fact shifted the results of true positive/true negative to a higher error and to a reduced the test sensitivity. Other reasons may be caused by a different affinity of species-specific antibodies [33] of camels *vs.* those of bovines as well as the IgG deficiency of camels [37,38], which may explain the fluctuations of the area under the curve and the different Se values as shown in Figure. 3.3C. Truly negative and positive controls will need and have

a positive effect on future validations. In this study, 7 true positive and 29 true negative camel serum samples were confirmed by real time PCR as an indication of a true positive and true negative for ROC analysis.

Hence, ROC analysis as a traditionally risk prediction model has shown that this cELISA can be used to detect anti-*Anaplasma* antibodies in camel sera and to estimate the preliminary prevalence of anaplasmosis in camels. At present, it might already be used in early warning systems and to monitor changes in the activity of the disease. Considering the increasing importance of camels in the future it therefore makes sense to further validate the WMRD *Anaplasma* cELISA kit for use in camels. It has to be stressed that there does not exist other studies to compare these in silico findings. Simulation would have been more effective and realistic if data from other studies were available. Chi square analyses revealed that the domain and origin of animals are the only significant risk factor (domains: $\chi(2) = 41.8$, p value = < 0.001 and origin: $\chi(2) = 42.56$, p value = < 0.001). These may be due to the lack of a proper distribution of health policies in most of the areas and the origin of animals as a source of disease transmission through the importation.

In this study, bovine serum and bovine controls serum provided with this commercial cELISAv2 kit confirms that cELISA can be used with confidence to determine %I and to confirm the presence or absence of anti-*Anaplasma* antibody in camel serum. The results of this study proved that cELISAv2 kit was validated for the detection of anti-*Anaplasma* antibody in camels. The cELISA used in this study appeared to meet the criteria for use in diagnosing anaplasmosis and screening in camels for the presence of the *Anaplasma*-specific antibody.

Alternative (*in silico*) validation techniques and preliminary prevalence studies are the first steps towards control of neglected anaplasmosis in the generally untended but increasingly important farm animal camel.

It can be assumed that raising of society awareness especially in veterinarians and animal owners will significantly contributed to our understanding of anaplasmosis in Egypt.

Author Contributions: Data curation, O.P.; formal analysis, O.P.; investigation, O.P. and K.M.-S.; methodology, O.P. and K.M.-S.; supervision, H.E.-A., U.R., H.N. and K.M.-S.; writing original draft, O.P. and H.N.; writing—review and editing, O.P., H.E.-A., U.R., H.N. and K.M.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by German Federal Foreign Office, Germany grant number [AA-OR-12-370.43 BIOS FLI EGY] and the APC was funded by Institute of Bacterial Infections and Zoonoses, Friedrich-Loffler-Institut, Jena, Germany.

Acknowledgments: The authors thank co-workers at the Friedrich-Loeffler-Institut for their cooperation and technical assistance. This research work was financially supported by the International Research Project as part of the "German Biosecurity Program" funded by Federal Foreign Office, Germany.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Herbison, L.; Frame, G.W. Encyclopedia Britannica: Camel. Availabe online: https://www.britannica.com/animal/camel (accessed on 27 February 2020).
2. CAPMAS. Central Agency for Public Mobilization and Statistics Egypt. Availabe online: http://www.capmas.gov.eg/HomePage.aspx (accessed on 27 February 2020).
3. Azmat, M.; Ijaz, M.; Farooqi, S.H.; Ghaffar, A.; Ali, A.; Masud, A.; Saleem, S.; Rehman, A.; Ali, M.M.; Mehmood, K.; et al. Molecular epidemiology, associated risk factors, and phylogenetic analysis of anaplasmosis in camel. *Microb. Pathog.* **2018**, *123*, 377–384, doi:10.1016/j.micpath.2018.07.034.
4. Dahlgren, F.S.; Mandel, E.J.; Krebs, J.W.; Massung, R.F.; McQuiston, J.H. Increasing incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in the United States, 2000–2007. *Am. Soc. Trop. Med. Hyg.* **2011**, *85*, 124–131, doi:10.4269/ajtmh.2011.10-0613.

5. Constable, P.D.; Hinchcliff, K.W.; Done, S.H.; Grünberg, W. Diseases of the hemolymphatic and immune systems. In *Veterinary Medicine*, 11th ed.; Saunders Ltd.: London, UK, 2017; doi:10.1016/B978-0-7020-5246-0.00011-5, pp. 1–2278.
6. Sudan, V.; Sharma, R.L.; Borah, M.K. Subclinical anaplasmosis in camel (*Camelus dromedarius*) and its successful therapeutic management. *J. Parasit. Dis.* **2014**, *38*, 163–165, doi:10.1007/s12639-012-0206-0.
7. Abou El-Naga, T.R.; Barghash, S.M. Blood parasites in camels (*Camelus dromedarius*) in Northern West Coast of Egypt. *J. Bacteriol. Parasitol.* **2016**, *07*, doi:10.4172/2155-9597.1000258.
8. Bastos, A.D.S.; Mohammed, O.B.; Bennett, N.C.; Petevinos, C.; Alagaili, A.N. Molecular detection of novel *Anaplasmataceae* closely related to *Anaplasma platys* and *Ehrlichia canis* in the dromedary camel (*Camelus dromedarius*). *Vet. Microbiol.* **2015**, *179*, 310–314, doi:10.1016/j.vetmic.2015.06.001.
9. Schotthoefer, A.M.; Meece, J.K.; Ivacic, L.C.; Bertz, P.D.; Zhang, K.; Weiler, T.; Uphoff, T.S.; Fritsche, T.R. Comparison of a real-time PCR method with serology and blood smear analysis for diagnosis of human anaplasmosis: Importance of infection time course for optimal test utilization. *J. Clin. Microbiol.* **2013**, *51*, 2147–2153, doi:10.1128/jcm.00347-13.
10. Madruga, C.R.; Marques, A.P.C.; Leal, C.R.B.; Carvalho, C.M.E.; Araújo, F.R.; Kessler, R.H. Evaluation of an enzyme-linked immunosorbent assay to detect antibodies against Anaplasma marginale %J Pesquisa Veterinária Brasileira. *Pesqui. Veterinária Bras.* **2000**, *20*, 109–112.
11. Greiner, M.; Pfeiffer, D.; Smith, R.D. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Prev. Vet. Med.* **2000**, *45*, 23–41, doi:10.1016/S0167-5877(00)00115-X.
12. OIE. Chapter 1.1.8. Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018*; OIE: Paris, France, 2019. Availabe online: http://www.oie.int/standard-setting/terrestrial-manual/access-online/ (accessed on 27 February 2020).
13. Pontius, R.; Si, K. The total operating characteristic to measure diagnostic ability for multiple thresholds. *Int. J. Geogr. Inf. Sci.* **2014**, *28*, 570–583, doi:10.1080/13658816.2013.862623.
14. Perkins, N.J.; Schisterman, E.F.; Vexler, A. Receiver operating characteristic curve inference from a sample with a limit of detection. *Am. J. Epidemiol.* **2007**, *165*, 325–333, doi:10.1093/aje/kwk011.
15. Mason, K.L.; Gonzalez, M.V.; Chung, C.; Mousel, M.R.; White, S.N.; Taylor, J.B.; Scoles, G.A. Validation of an improved *Anaplasma* antibody competitive ELISA for detection of *Anaplasma ovis* antibody in domestic sheep. *J. Vet. Diagn. Investig.* **2017**, *29*, 763–766, doi:10.1177/1040638717709494.
16. Scoles, G.A.; Goff, W.L.; Lysyk, T.J.; Lewis, G.S.; Knowles, D.P. Validation of an *Anaplasma marginale* cELISA for use in the diagnosis of *A. ovis* infections in domestic sheep and *Anaplasma* spp. in wild ungulates. *Vet. Microbiol.* **2008**, *130*, 184–190, doi:10.1016/j.vetmic.2007.12.020.
17. Classen, D.C.; Morningstar, J.M.; Shanley, J.D. Detection of antibody to murine cytomegalovirus by enzyme-linked immunosorbent and indirect immunofluorescence assays. *J. Clin. Microbiol.* **1987**, *25*, 600–604.
18. Pan, A.A.; Rosenberg, G.B.; Hurley, M.K.; Schock, G.J.; Chu, V.P.; Aiyappa, A. Clinical evaluation of an EIA for the sensitive and specific detection of serum antibody to *Trypanosoma cruzi* (Chagas' disease). *J. Infect. Dis.* **1992**, *165*, 585–588.
19. Lardeux, F.; Torrico, G.; Aliaga, C. Calculation of the ELISA's cut-off based on the change-point analysis method for detection of *Trypanosoma cruzi* infection in Bolivian dogs in the absence of controls. *Memórias Inst. Oswaldo Cruz* **2016**, *111*, 501–504, doi:10.1590/0074-02760160119.
20. Frey, A.; Di Canzio, J.; Zurakowski, D. A statistically defined endpoint titer determination method for immunoassays. *J. Immunol. Methods* **1998**, *221*, 35–41, doi:10.1016/S0022-1759(98)00170-7.
21. Elhariri, M.D.; Elhelw, R.A.; Hamza, D.A.; Soliman, D.E. Molecular detection of *Anaplasma marginale* in the Egyptian water bufaloes (*Bubuloes bubalis*) based on major surface protein 1α. *J. Egy. Soc. Parasitol.* **2017**, *47*, 247–252.
22. Fereig, R.M.; Mohamed, S.G.A.; Mahmoud, H.; AbouLaila, M.R.; Guswanto, A.; Nguyen, T.T.; Ahmed Mohamed, A.E.; Inoue, N.; Igarashi, I.; Nishikawa, Y. Seroprevalence of *Babesia bovis*, *B. bigemina*, *Trypanosoma evansi*, and *Anaplasma marginale* antibodies in cattle in southern Egypt. *Ticks Tick-Borne Dis.* **2017**, *8*, 125–131, doi:10.1016/j.ttbdis.2016.10.008.
23. Loftis, A.D.; Reeves, W.K.; Szumlas, D.E.; Abbassy, M.M.; Helmy, I.M.; Moriarity, J.R.; Dasch, G.A. Rickettsial agents in Egyptian ticks collected from domestic animals. *Exp. Appl. Acarol.* **2006**, *40*, 67–81, doi:10.1007/s10493-006-9025-2.

24. Loftis, A.D.; Reeves, W.K.; Szumlas, D.E.; Abbassy, M.M.; Helmy, I.M.; Moriarity, J.R.; Dasch, G.A. Surveillance of egyptian fleas for agents pf public health significance: *Anaplasma*, *bartonella*,*coxiella*, *ehrlichia*. *Am. Soc. Trop. Med. Hyg.* **2006**, 75, 41–48.
25. Loftis, A.D.; Reeves, W.K.; Szumlas, D.E.; Abbassy, M.M.; Helmy, I.M.; Moriarity, J.R.; Dasch, G.A. Population survey of Egyptian arthropods for rickettsial agents. *Ann. N. Y. Acad. Sci.* **2006**, *1078*, 364–367, doi:10.1196/annals.1374.072.
26. Radwan, M.E.; Ali, A.; Abd el Hamied, O. Epidemiological Studies ,Molecular Diagnosis of *Anaplasma marginale* in Cattle and Biochemical Changes Associated with it in Kaliobia Governorate. *Glob. Adv. Res. J. Med. Med Sci.* **2013**, *1*, 46–49, doi:10.12691/ajidm-1-3-2.
27. Barghash, S.M.; Hafez, A.A. Molecular detection of pathogens in ticks infesting camels in Matrouh governorate, Egypt. *J. Bacteriol. Parasitol.* **2016**, *07*, 1–7, doi:10.4172/2155-9597.1000269.
28. Ghafar, M.W.; Amer, S.A. Prevalence and first molecular characterization of *Anaplasma phagocytophilum*, the agent of human granulocytic anaplasmosis, in Rhipicephalus sanguineus ticks attached to dogs from Egypt. *J. Adv. Res.* **2012**, *3*, 189–194, doi:10.1016/j.jare.2011.08.002.
29. Salm, F.F.; Younis, E.E.; Hegazy, N.M.; El-Sawalhy, A.A. Epidemiological studies on bovine anaplasmosis. *Bull. Anim. Health Prod. Afr.* **2011**, *59*, 179–189.
30. Klemmer, J.; Njeru, J.; Emam, A.; El-Sayed, A.; Moawad, A.A.; Henning, K.; Elbeskawy, M.A.; Sauter-Louis, C.; Straubinger, R.K.; Neubauer, H.; et al. Q fever in Egypt: Epidemiological survey of *Coxiella burnetii* specific antibodies in cattle, buffaloes, sheep, goats and camels. *PLoS ONE* **2018**, *13*, e0192188, doi:10.1371/journal.pone.0192188.
31. VMRD. Product Catalog. Availabe online: https://www.vmrd.com/core/files/vmrd/uploads/files/VMRD%20Catalog_4_2_18.pdf (accessed on 27 February 2020).
32. Scoles, G.A.; Ueti, M.W.; Palmer, G.H. Variation among geographically separated populations of dermacentor andersoni (Acari: *Ixodidae*) in midgut susceptibility to *Anaplasma marginale* (Rickettsiales: *Anaplasmataceae*). *J. Med. Entomol.* **2005**, *42*, 153–162, doi:10.1093/jmedent/42.2.153.
33. Davies, C. Immunoassay Performance Measures. In *The Immunoassay Handbook*, 4th ed.; Wild, D., Ed.; Elsevier: Oxford, UK, 2013; doi:10.1016/B978-0-08-097037-0.00003-8, pp. 11–26.
34. Theodorsson, E.; Magnusson, B.; Leito, I. Bias in clinical chemistry. *Bioanalysis* **2014**, *6*, 2855–2875, doi:10.4155/bio.14.249.
35. Perez, A.M. Past, present, and future of veterinary epidemiology and economics: One health, many challenges, no silver bullets. *Front. Vet. Sci.* **2015**, *2*, 60–60, doi:10.3389/fvets.2015.00060.
36. Napp, S.; Chevalier, V.; Busquets, N.; Calistri, P.; Casal, J.; Attia, M.; Elbassal, R.; Hosni, H.; Farrag, H.; Hassan, N.; et al. Understanding the legal trade of cattle and camels and the derived risk of Rift Valley Fever introduction into and transmission within Egypt. *PLoS Negl. Trop. Dis.* **2018**, *12*, doi:10.1371/journal.pntd.0006143.
37. Agarwal, S.; Cunningham-Rundles, C. Assessment and clinical interpretation of reduced IgG values. *Ann. Allergy Asthma Immunol.* **2007**, *99*, 281–283, doi:10.1016/S1081-1206(10)60665-5.
38. IMGT. *Camelidae* IgG Antibodies. Availabe online: http://www.imgt.org/IMGTbiotechnology/Camel_IgG.html (accessed on 18 July 2019).

General discussion

Anaplasma spp. are a group of obligate intracellular Gram negative bacteria that cause diseases of the hemolymphatic and immune system (Constable et al., 2017; Silaghi et al., 2017). Clinical symptoms vary depending on the pathogen and animal host but include thrombocytopenia, hemolytic anemia, abortion and death (Constable et al., 2017). The OIE recommended serological test, the cELISA, is based on the detection of antibodies against manbrane surface proteins of *A. marginale* (OIE, 2019). *Anaplasma* spp. infection can be confirmed by real time PCR. These methods have suitable sensitivity and specificity. Cross species transmission may occur among the various animal hosts through vectors i.e. ticks and is favoured by mismanagement in farm animals. Thus, presence of Anaplasmae may be considered an indicator for the quality of farm management in farm animals. Because of the way of transmission, the eradication of anaplasmosis requires accurate countermeasure programs. Sometimes eradication is only possible if infected animals are replaced because of transplacentaly transmission in chronically-infected animals (Henniger et al., 2013).

Although, North Africa and the Middle East have predominantly hot desert or hot semi-arid climates (Waha et al., 2017) which are not suitable for the tick vectors, the eradication of disease is apparently not feasible due to the lifestyle of ticks and management practices. Dantas-Torres reported on the factors influencing tick behavior such as stability of distribution during climate change, role of 'tropic cascades' i.e. an ecological phenomen caused by changes in the population of predators and prey in the feeding relationships or 'food web' as a start point of ecological processes. These phenomens can increase aggressiveness of ticks or multi-hosting, and last but not least the role of host population dynamics. As an example, the transmission dynamics of *B. burgdorferi* was considerably altered with fluctuations of small-mamel host populations (Dantas-Torres, 2015). Control-, suitable countermeasures- or eradication programs can only be successful if keeping in mind the life cycle, the interface of agent and host and the production system in place (Dantas-Torres, 2015). Especially factors that have influence on the distribution of pathogens and are disregarded in countermeasures programs have to be identified. To understand these dynamics, this work tries to collect data from as many sources as available between 1959 and 2019. Interface, territorial context and host population density, nomadic life style and obvious mismanagement were identified as main drivers of infections. There was a lack of a holistic strategy for the eradication of anaplasmosis identified in the study region as well. Data comparison showed the significant increase in numbers of farm animals during this period and intensive breeding (Table 1.4, chapter 1). A comparison of transcontinental states of this corridor, eg. Egypt and Yemen, reveals enormous differences in the development status of epidemiology research and legislation (Tables 1.5-1.8, chapter 1).

Cooperation of international or national reference centers and universities on the exchange of knowledge is growing.

As a result, development of diagnostic methods and phylogenetic analysis was intensified as already stressed by OIE. This intensification of research is more prevalent in the western part of the corridor due to cooperations of European and North African researchers in various projects. An increasing trend of statistical analysis in scientific papers is obvious (Tables 1.5-1.8 in chapter 1) but metadata are available only for a few of them. Tracing of information was possible based on accessible and available data for few countries (i.e. *Anaplasma* was mentioned in Egyptian state reports since 1966) but a consistent history of official information is missing. Description of the role of veterinary organizations or agricultural ministries as head of the control, monitoring or eradication programs of anaplasmosis is missing. Apparently, recognition and awareness raising for anaplasmosis in public veterinary health is not in the political focus of most countries of the corridor.

The genetic similarity of Anaplasmae of different countries of this region was striking. An intensive trade relationship between 1991-2017 between these countries was also demonstrated via World Integrated Trade Solution (WITS) (Fig. 1.1, capter 1). Thus, it can be assumed that this corridor is indeed an important turntable for the intercontinental spread of anaplasmosis. The life style of Bedoin tribes of the region and the most important Islamic festival of sacrifice, “Eid al-Adha”, and the inevitable movment of slaughter animals also could be reasons for an evenly distribution. Both factors are a great challenge for veterinary- and public health due to silent transmission by carrier animals. In summary, it can be concluded that local motility and intercontinental trade are the important drives for anaplasmosis. It is an obvious need that these hypotheses have to be verified statistically now, whereas spread of diseases through human mobility (Meloni et al., 2011), human action (Lindahl and Grace, 2015) and human behavior (Funk et al., 2010) is a known fact.

Anaplasma marginale, *A. centrale*, *A. ovis*, and *A. phagocytophilum* were identified to be endemic in Algeria, Morroco and Tunisia. *A. bovis* and *A. platy* were present in Algeria and *A. bovis* was found in Tunisia. Moving East *A. marginale*, *A. centrale* and *A. phagocytephilum* were found in Egypt, *A. marginale*, *A. platy* and *A. ovis* in Sudan, *A. ovis* and *A. platy* in State of Palestine, *A. phagocytophilum* in Jordan, *A. ovis* in Iraq, *A. platy* in Qatar and *A. phagocytophilum* and *A. ovis* in Saudi Arabia. With the exception of Iraq and Sudan due to instability of government, it can be supposed that the lack of data is connected to the ignorance of involved public health authorities. The impact of *Anaplasma* spp. infection on livestock production is well known. The unavailability of data from Lebanon, Syrian Arab Republic and State of Palestine may be due to instability of the governmental structures. The reasons for the missing of data for Oman, Kuwait, and United Arab Emirates are not obvious. A comprehensive description of the epidemiologic situation is not possible.

Egypt as a transcontinental country with a central position in this corridor may serve as an interface for information from is region. However, a surveillance study involving all governorates of Egypt is missing. A preliminary screening of the sera of cattle from 24 governorates using commercial cELISA and real time PCR kits revealed acceptable results. Cattle of 17 governorates were serological positive. Molecular assays confirmed the presence of *Anaplasma* spp. in 12 governorates with 2.89-25% positive sera. 25% of the sera from Port Said and Suez haboured DNA highlighting the role of this passage and for intercontinental spread. Although frequent outbreaks can be traced in the state reports, they are not reflected in the scientific literature as was expected. The reasons of this disregarding is not clear. It can be supposed that the well known transmission way via arthropods and susceptibility of the pathogens to antibiotics lead to little attention of scientists on anaplasmosis. A lack of funding of basic and applied science corresponds to the lack of knowledge, awareness and control.

Silent carriers, transplacental transmission and missmanagement are the drivers of the disease. Livestock dehorning leads to spread of the disease within farms due to disregarding disinfection of tools. Thus, raising the knowledge of farm owners will play an important role to control this disease. Implementation of training programs to raise the public awareness could be an effective way to reduce the economic impact of anaplasmosis. Analysis of the genome of *Anaplasma* spp. suggests that transmission is connected to ani mal movement e.g. international trade or nomadism. Local solutions for the “local” probl ems i.e. habitat and religious practices need to be respected and need to be substantial and integral elements of any control.

A general lack of diagnostic means for the farm animal camel is obvious. Use of the commercial competitive *Anaplasma* ELISA v2 (Veterinary Medical Research and Development Inc., Pullman, WA, USA) for camels proved the presence of anaplasmosis in farm animals. The cELISA had to be adopted before use accordingly as it was done previously for use in sheep (Mason et al., 2017; Scoles et al., 2008). A method was developed for preliminary monitoring of anaplasmosis combining in silico methods and available laboratory results. Scatterploting of mean optical densities of cattls vs. camels cELISA results (Figure 3.4 in chapter 3) showed a clear correlation. The true extend scatterplot (Figure 3.4 in chapter 3) showed evidence for unknown parametrers and has impact on the results of serological tests. Cutoff value of 0.42 resulted in highest sensitivity and specificity. A simulation based on 2,300 samples with 10 percent false positive/negative proved a sufficiant specificity. Repeating the experiment by getting rid of problems caused by negative controls will result in a better performance of the test. Screening then showed a totally of 34.1% positive sera in camel populations of Egypt.

References

Dantas-Torres, F., 2015. Climate change, biodiversity, ticks and tick-borne diseases: The butterfly effect. International journal for parasitology. Parasites and wildlife 4, 452-461.

Constable, P.D., Hinchcliff, K.W., Done, S.H., Grünberg, W. 2017. Chapter 11-Diseases of the Hemolymphatic and Immune Systems, In: Veterinary Medicine (Eleventh Edition). W.B. Saunders. doi: https://doi.org/10.1016/B978-0-7020-5246-0.00011-5.

Dantas-Torres, F., 2015. Climate change, biodiversity, ticks and tick-borne diseases: The butterfly effect. International journal for parasitology. Parasites and wildlife 4, 452-461.

Funk, S., Salathé, M., Jansen, V.A.A., 2010. Modelling the influence of human behaviour on the spread of infectious diseases: a review. J R Soc Interface 7, 1247-1256.

Henniger, T., Henniger, P., Grossmann, T., Distl, O., Ganter, M., von Loewenich, F.D., 2013. Congenital infection with Anaplasma phagocytophilum in a calf in northern Germany. Acta Vet Scand 55, 38.

Lindahl, J.F., Grace, D., 2015. The consequences of human actions on risks for infectious diseases: a review. Infect Ecol Epidemiol 5, 30048-30048.

Mason, K.L., Gonzalez, M.V., Chung, C., Mousel, M.R., White, S.N., Taylor, J.B., Scoles, G.A., 2017. Validation of an improved Anaplasma antibody competitive ELISA for detection of Anaplasma ovis antibody in domestic sheep. J Vet Diagn Invest 29, 763-766.

Meloni, S., Perra, N., Arenas, A., Gómez, S., Moreno, Y., Vespignani, A., 2011. Modeling human mobility responses to the large-scale spreading of infectious diseases. Scientific Reports 1, 62.

OIE 2019. Chapter 3.4.1. Bovine anaplasmosis. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, Paris, France). http://www.oie.int/standard-setting/terrestrial-manual/access-online/.

Scoles, G.A., Goff, W.L., Lysyk, T.J., Lewis, G.S., Knowles, D.P., 2008. Validation of an Anaplasma marginale cELISA for use in the diagnosis of A. ovis infections in domestic sheep and Anaplasma spp. in wild ungulates. Vet Microbiol 130, 184-190.

Silaghi, C., Santos, A.S., Gomes, J., Christova, I., Matei, I.A., Walder, G., Domingos, A., Bell-Sakyi, L., Sprong, H., von Loewenich, F.D., Oteo, J.A., de la Fuente, J., Dumler, J.S., 2017. Guidelines for the Direct Detection of Anaplasma spp. in Diagnosis and Epidemiological Studies. Vector Borne Zoonotic Dis 17, 12-22.

Waha, K., Krummenauer, L., Adams, S., Aich, V., Baarsch, F., Coumou, D., Fader, M., Hoff, H., Jobbins, G., Marcus, R., Mengel, M., Otto, I.M., Perrette, M., Rocha, M., Robinson, A., Schleussner, C.F., 2017. Climate change impacts in the Middle East and Northern Africa (MENA) region and their implications for vulnerable population groups. Regional Environmental Change 17, 1623-1638.

Summary

Overview of Anaplasmosis in Arab Countries in North Africa and the Middle East, and Optimizing a commercial c-ELISA for Camels

Anaplasmosis is a tick-borne disease with a great economic importance for cattle farming that causes disorders of the hemolymphatic and immune system. It is distributed worldwide in tropical and sub-tropical countries. The economic impact of the disease is significant for animal welfare and public health. This work provides comprehensive information on anaplasmosis through a literature review of 19 Arab countries in the North Africa and the Middle East. Screening of cattle sera from Egypt was performed using commercial cELISA and real time PCR. Validation of a 'bovine' cELISA for use in camel sera, a ROC curve analysis was used to estimate the cutoff value, sensitivity and specificity.

The number of anaplasmosis positive samples using molecular assays ranged from 4.4-61.7% in cattle and sheep in Algeria; 14.08-67.37% in cattle in Egypt; 7.5- 71% in dogs and small ruminants in Morocco; 0.6-69.6% in cattle and goats in Tunisia; 6.1-24.4% in cattle and dogs in Sudan; 62.6% in sheep in Iraq; 39.5% in dogs in Jordan; 1.6% in dogs in Qatar and 15.5-38.1% in small ruminants in Saudi Arabia. In Saudi Arabia, a high number of anaplasmosis positive samples from slaughter animals was noticed.

In Egypt, cattle sera revealed a seroprevalence of 18.46% (CI: 15.8-21.4%) and 5.3% (CI: 3.8-7.1%) using cELISA or real-time PCR. Some of the sera (3.95%) were also positive for *C. burnetii*-specific antibodies. The best cutoff value of cELISA was calculated to be 0.42 ($p < 0.001$) for camels sera whereas this value for cattle sera was 0.4022. Trace immanence of bovine ELISA vs. camel ELISA methods was shown as scatterplot. Prevalence in camels was analysed finally with a cutoff of 0.42. In New Valley Delta and Eastern Desert domain prevalences of 47.4% und 46.4% were found, respectively. The simulation for 2,300 generated data with 10% error allowed resulted in 97.8% specificity.

The initial aims of the thesis i.e. to write a comperhensive review fo anaplasmosis for Northen Africa and the Near East, to adopt a ‚bovine' cELISA for use in camel sera and a preliminary study seroprevalence study for anaplasmosis in Egypt were succesfully fullfilled.

Zusammenfassung

Überblick über Anaplasmose in arabischen Ländern in Nordafrika und im Nahen Osten und Optimierung eines kommerziellen c-ELISA für die diagnostische Verwendung bei Kamelen

Anaplasmose ist eine Zecken-übertragene Erkrankung und eine Zoonose mit großer ökonomischer Bedeutung für die Rinderhaltung. Sie kann Störungen des hämolymphen und des Immunsystems verursachen und ist weltweit überwiegend in tropischen und subtropischen Ländern verbreitet.

Diese Arbeit enthält umfassende Informationen zur Anaplasmose anhand eines Literatur-reviews in 19 Ländern Nordafrikas und des Nahen Ostens. Experimentell wurde eine Orientierungsstudie zur Prävalenz von Anaplasmose mittels Rinderseren aus Ägypten unter Verwendung kommerzieller serologischer (cELISA) und molekularer Kits (real time PCR Kit) durchgeführt. Zusätzlich wurden auch Kamelseren utersucht. Zur Anwendung des für Rinder entwickelten cELISAs zur Untersuchung von Kamelseren wurde eine Optimierung mittls Operationscharakteristik (ROC-Kurve) vorgenommen, um den Grenzwert, Sensitivität und Spezifität abzuschätzen. Der beste ‚Cutoff'-Wert für Kamelseren liegt bei 0,42 (p-Wert <0,001), während dieser Wert bei Rindern 0,4022 betrug. Für Kamele wurde die Prävalenz abschließend mit einem Cutoff von 0,42 analysiert. Eine Simulation für 2300 generierte Daten mit 10% Fehler ergab eine Spezifität von 97.8%. Es wurde festgestellt, dass das New Valley Delta und die Eastern Desert Domain eine hohe Anaplasmose Prävalenz von 47.4% und 46.4% aufweisen. Eine ausführliche Auswertung öffentlich verfügbarer Litratur und von Staatlichen Quellen ergab eine mangelhafte Datenlage zur Epidemiologie und zur Perzeption der Anaplasmose im Allgemeinen in 19 Staaten Nord Afrikas und des Nahen Ostens. Die Anzahl von Anlass bezogenen, Anaplasmose-positiven Proben lag z.B. in Algerien zwischen 4,4 und 61,7% bei Rindern und Schafen, 14,08-67,37% bei Rindern in Ägypten, 7,5- 71% bei Hunden und kleinen Wiederkäuern in Marokko, 0,6-69,6% bei Rindern und Ziegen in Tunesien, 6,1-24,4% bei Rindern und Hunden im Sudan, 62,6% bei Schafen im Irak, 39,5% bei Hunden in Jordanien, 1,6% bei Hunden in Katar und 15,5-38,1% bei kleinen Wiederkäuern in Saudi-Arabien. In Saudi-Arabien wurde eine hohe Anzahl an Anaplasmose-positiven Proben bei geschlachteten Schafen festgestellt.

Eigene Untersuchungen von Rinderseren aus ägyptitichen Governourates ergaben eine Seroprävalenz von 18,46% (CI: 15,8-21,4%) und 5.3% (CI: 3.8-7.1%) mittels cELISA bzw. real time PCR. Einige der Seren (3.95%) waren ebenfalls positiv für *C. burnetii*-spezifische Antikörper.

List of published articles

1. Parvizi O, El-Adawy H, Melzer F, Roesler U, Neubauer H, Mertens-Scholz K. Seroprevalence and Molecular Detection of Bovine Anaplasmosis in Egypt. *Pathogens*. 2020; 9(1):E64. Published 2020 Jan 16. http://doi:10.3390/pathogens9010064
2. Parvizi O, El-Adawy H, Roesler U, Neubauer H, Mertens-Scholz K. Performance Analysis of *Anaplasma* Antibody Competitive ELISA Using the ROC Curve for Screening of Anaplasmosis in Camel Populations in Egypt. *Pathogens*. 2020; 9(1), 64; https://doi.org/10.3390/pathogens9010064
3. O. Parvizi, K.O. Akinyemi, U. Roesler, H. Neubauer & K. Mertens-Scholz. Retrospective study of anaplasmosis in countries of North Africa and the Middle East. Scientific and Technical Review. 2021; 39 (3); https://www.oie.int/en/what-we-do/publications/scientific-and-technical-review/

Acknowledgements

"Be happy for this moment. This moment is your life."

(Omar Khayyam)

This research presented in this dissertation is part of the German Biosecurity Program, which is funded by Federal Foreign Office, Germany in collaboration with Friedrich-Loeffler-Institut, Institute of Bacterial Infections and Zoonoses, Jena, Germany.

My special thanks go to my supervisor, Prof. Dr. Heinrich Neubauer, for the opportunity to work at the Friedrich-Loeffler-Institut, Jena, Germany. I would like to thank him for his inspiration, discussion and patience to correct my manuscript. My special thanks go to my supervisor Prof. Rösler and his Institute for supporting this work. Many thanks also to Dr. Katja Mertens-Scholz, the head of working group of Rictsiology and NRL for Q-fever at FLI, Jena and her crew and Nadine Engelhardt for her active support. My special thanks go to Dr. Hosny El Adawy for his countinioued support. My special thanks go to PD Dr. Angela Berndt, Dr. Claudia Gerlach, and Dr. rer. nat. Michael Boehringer for their discussions and the opportunities to exchange my thoughts. I would like to thank Maria-Christina Haase for her suggestions and ideas.

Appendix A

Material and methods

Cross-reactivity prediction using in silico techniques

Cross-reactivity defines the type of antigen variation that measures the degree of antigens similarity to the immune system (Frank, 2002). In silico techniques such as Multiple Alignment using Geneious® 10.2.3 and NCBI Blast involving BLASTP, PSI-BLAST and DELTA BLAST (Altschul et al., 1997) were used to predict any cross reactivity in cELISA results based on similarity between msp5 antigen of *A. marginale* (accession no. M93392: AAB02878.1) and other antigens of potential pathogens.

Results

In silico analysis

Multiple alignment of *A. marginale* msp5 (accession no. M93392: AAB02878.1) and CB (accession no. CP000733) found almost 30% of similarity at the gene level but only 17% at the protein level. Using 'protein query' or 'domain matching', BLASTP searches showed all (n=100) homologous sequences deposited for the family *Anaplasmataceae*. The algorithms found homology of *A. phagocytophilum* and *Ehrlichia ruminantium* with following values [WP_060757743.1, 63.94%, 2e-92, 98%, 278] and [GAT78011.1, 49.28%, 9e-68, 98%, 216] for parameters "no. accession, identity, e-value, query cover, score" in each bracket, respecttively. PSI-BLAST revealed homologies of *A. ovis*, *A. centrale, A. phagocytophilum, E. canis*, *E. ruminantium* with values [WP_075138732.1, 93.33%, 3e-135, 100%, 387], [WP_012880973.1., 91.43%, 4e-131, 100%, 376], [ABP65332.1, 68.32%, 1e-44, 48%, 153], [WP_011304280.1, 51.21%, 2e-71, 98%, 225], [GAT78011.1, 49.28%, 9e-68, 98%, 216], respectively. DELTA-BLAST runs with the exclude *Anaplama* option (Entrez Query: NOT *Anaplasma*) identified 500 homologue sequences, primarily derived from α-proteobacteria found in the environment. The results of BLASTP in detail were: *A. marginale* (51%), *A. phagocytophilum* (24%), *Ehrlicha* spp. (4%), *E. ruminantium* (7%), *A. ovis* (2%), *A. centrale* (2%), *E. canis* (1%), Anaplasma spp. (5%), *E. chaffeensis* (1%), *E. minasensis* (1%), *E. muris* (1%) and candidatus *Neoehrlichia lotoris* (1%).

Discussion

In silico analyses can prove the specificity and sensitivity antigens used in cELISAs. These test parameters can be influenced by homologous antigens of other pathogens such as *Ehrlichia ruminantium* considering *A. marginale* msp5. Thus, results have to be interpreted carefully. Due to the available matrix, low quality of sequences analysis and low quality of sequences in the databank no potentially cross-reactive structures could be derived in this study.

Selbstständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbstständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen Anspruch genommen habe.

Berlin, den 07.10.2020 Omid Parvizi

Druck: Mensch und Buch Verlag Berlin