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Anaplasma spp. in North Africa: A review on molecular epidemiology, associated risk factors and genetic characteristics

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ABSTRACT

The genus *Anaplasma* belonging to the Anaplasmataceae family (order Rickettsiales) comprises obligate intracellular Gram-negative bacteria of veterinary and public health importance. Six species and five types of strains genetically related are currently assigned to the genus *Anaplasma* including *Anaplasma marginale, A. centrale, A. bovis, A. phagocytophilum, A. ovis* and *A. platys* as classified species, and *"A. capra", A. odocolei* sp. nov., *A. phagocytophilum*-like 1 (*Anaplasma* sp.-Japan), *A. phagocytophilum*-like 2 (*Anaplasma* sp.-China) and *A. platys*-like (also named *Candidatus* Anaplasma camelii) as unclassified strains. Most of these *Anaplasma* species and strains have been molecularly identified in several animal and/or tick species in the north of Africa. The aim of this review is to summarize the current knowledge about molecular epidemiology, associated risk factors and genetic diversity of *Anaplasma* species and related strains infecting animals and/or their incriminated tick vectors in North Africa. All these data should be considered when establishing of common management and control programs for anaplasmosis infecting humans and different animal species in North African countries.

1. Introduction

According to the United Nations, countries belonging to North Africa are Morocco (including Western Sahara as territory), Algeria, Tunisia, Libya, Egypt and Sudan (https://unstats.un.org/unsd/methodology/m49/). This northernmost region of Africa is limited by the Mediterranean Sea in the north, the Atlantic Ocean in the west and the Sahara in the south. Except Sudan, the climate is Mediterranean with four seasons which are distinguished by a hot summer with little rain and a winter often soft and rainy. For the most part of Sudan, the climate is tropical with two seasons, a summer often hot and rainy and a cold winter with little rainfall.

The North African ecosystem represents a favorable ecology for several tick species which can transmit numerous pathogens, including protozoan, viral, and bacterial agents (Gharbi and Darghouth, 2014). Among these, anaplasmosis is a tick-borne rickettsial disease caused by *Anaplasma marginale*, *A. centrale*, *A. phagocytophilum*, *A. bovis, A. ovis* and *A. platys* (Dumler et al., 2001; Rar and Golovljova, 2011; Atif, 2016). In addition, unclassified *Anaplasma* spp. have been reported. Indeed, a newly *Anaplasma* species named *Anaplasma odocolei* sp. nov. was isolated from captive white-tailed deer (*Odocoileus virginianus*) in the USA (Tate et al., 2013). Similarly, a novel *Anaplasma* species, with the proposed designation "*Anaplasma capra*", was identified in apparently healthy goats in China and has also been recognized as a zoonotic pathogen (Li et al., 2015a,b). In Japan, a potentially novel *Anaplasma* sp. (also named *A. phagocytophilum*-like 1 by Ben Said et al. (2015a, 2017b)) genetically related to *A. phagocytophilum* was detected in a sika deer (Ybañez et al., 2012). More recently in China, Kang et al. (2014) detected other strains genetically related to *A. phagocytophilum* (also named *A. phagocytophilum*-like 2 by Ben Said et al. (2015a, 2017b)) in *Hyalomma asiaticum* ticks infesting sheep and cattle, which differ from the Japanese strains (*A. phagocytophilum*-like 1) and from all other classified and unclassified *Anaplasma* strains. Furthermore, *A. platys*-like strains have been identified in neutrophils of cattle, sheep and goats (Zobba et al., 2014) and in cat platelets (Zobba et al., 2015).

In this last decade, most of these *Anaplasma* species and strains have been molecularly detected in several animal and tick species in North Africa (M'ghirbi et al., 2009, 2012, 2016; Awad et al., 2011; Ait Hamou et al., 2012; Renneker et al., 2013; Belkahia et al., 2014, 2015a,b, 2017a,b; El-Ashker et al., 2015; Ben Said et al., 2015a,b, 2017a,b; Dahmani et al., 2015; Aouadi et al., 2017; Ait Lbacha et al., 2017a,b; Elhamiani Khatat et al., 2017a; Rjeibi et al., 2017).

Furthermore, serological evidence has been provided for (i) *Anaplasma* spp. in dogs from Algeria (Azzag et al., 2015) and Morocco (Elhamiani Khatat et al., 2017a,b), (ii) *A. phagocytophilum* in Tunisian dogs, horses and camels (M'ghirbi et al., 2009, 2012; Ben Said et al., 2013, 2014), in Algerian dogs (Azzag et al., 2015) and in humans from Morocco (Elhamiani Khatat et al., 2016, 2017a), and (iii) *A. marginale*

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in cattle from Morocco (Verhulst et al., 1983; Ait Hamou et al., 2012), Egypt (Fereig et al., 2017) and Sudan (Salih et al., 2008, 2009).

In this review, we present a synthesis of knowledge about the molecular epidemiology of these *Anaplasma* species and related strains, and their associated risk factors in North Africa. Molecular characteristics and the phylogeny of classified and unclassified *Anaplasma* spp. strains infecting animals and ticks in North Africa are also described.

2. Molecular epidemiology and genetic characteristics

2.1. Anaplasma marginale

Anaplasma marginale, the most common etiologic agent of bovine anaplasmosis, is endemic worldwide especially in tropical and subtropical areas (Kocan et al., 2003). It has been described, for the first time by Theiler (1910) as “marginal points” that are round basophilic bodies (0.5–1 mm) frequently present on the periphery of erythrocytes on microscopic blood smear examination (Allison and Meinkoth, 2010). This bacterium causes a variety of clinical signs, including fever, weight loss, abortion, lethargy, icterus, and often death of the animals older than 2 years (Kocan et al., 2003). In North African countries, infection by this species has been investigated only in cattle and dromedaries. Belkahia et al. (2015b) failed to detect *A. marginale* in Tunisian camels. It can be postulated that other wild and domestic animal species like cattle could be infected with *A. marginale* in this area. This finding has been confirmed by several cross-sectional investigations on cattle (Ait Hamou et al., 2012; Belkahia et al., 2015a; El-Ashker et al., 2015; M’ghirbi et al., 2016) and similar infection rates with this pathogen have been estimated in cattle from Tunisia (24.7–25.4%), Morocco (21.9%) and Egypt (20.1%) during the period of tick activity (spring and summer) (Table 1 and Fig. 1). However, based on PCR method, lower *A. marginale* prevalences, estimated at 6.1% and 11.1%, have been recorded in cattle from Sudan and Algeria, respectively (Awad et al., 2011; Rjeibi et al., 2017). These results could be explained, in part, by a possible decrease of tick vectors’ infestation in hot rainy (Sudan) and winter (Algeria) seasons which were unfavorable to tick outbreaks (Awad et al., 2011; Rjeibi et al., 2017).

On the other hand, *A. marginale* DNA was detected in *Hyalomma excavatum* and *Rhipicephalus annulatus* ticks from Egypt by using polymerase chain reaction (PCR) and sequencing the *16S rRNA* and *msp5* partial genes, respectively (Loftis et al., 2006, Table 2). However, the vector competence of these tick species as well as their contribution to the transmission cycle of *A. marginale* remain to be investigated.

As shown in Table 2, the *msp4* gene has been used as a genetic marker for the characterization of *A. marginale* strains isolated from cattle in Tunisia and Algeria (Belkahia et al., 2015a, 2017a,b; M’ghirbi et al., 2016; Rjeibi et al., 2017). Notably, Belkahia et al. (2015a, 2017a,b) detected several *A. marginale* strains segregated in different clusters by the analysis of a partial sequence of the *msp4* gene (Table 2). This heterogeneity has been partially explained by the importation of live cattle and/or the dissemination of *A. marginale* infected ticks by migratory birds that have been demonstrated in several studies worldwide (Alekseev et al., 2001; Ogden et al., 2008; Hildebrandt et al., 2010; Kang et al., 2013). Results suggest multiple *A. marginale* introductions in Tunisia especially from Central Africa, Southern Europe and North American countries (Belkahia et al., 2015a, 2017a,b). Additionally, the presence of novel strains with a high-level diversity may be caused by a co-evolution process of *A. marginale* and its incriminated tick vectors found in Tunisia (Belkahia et al., 2015a). In fact, exploring the *msp4* polymorphism, de la Fuente et al. (2001c) showed the presence of a co-evolution between *A. marginale* and its tick vector, *Dermacentor variabilis*, in the USA.

2.2. Anaplasma centrale

Anaplasma centrale organisms look similar to *A. marginale* on blood smear examination but are located in the center of erythrocytes (Dumler et al., 2001; Allison and Meinkoth, 2010). *Anaplasma centrale,* which is less pathogenic than *A. marginale*, causes mild signs in cattle and is considered a naturally attenuated subspecies (Rar and Golovljova, 2011). This explains why it has been extensively used as a live vaccine against *A. marginale* in several countries (Kocan et al., 2010). The infection with *A. centrale* provides a significant and long-lasting protective immunity against highly virulent *A. marginale* strains infection (Kocan et al., 2003).

Until now, there have been no investigations on *A. centrale* in North Africa except in cattle and camels from Tunisia (Belkahia et al., 2015a,b, 2017a,b) and in cattle from Algeria (Rjeibi et al., 2017). *Anaplasma centrale* infection was detected in 15.1% and 39.4% of cattle from Tunisia and Algeria, respectively (Belkahia et al., 2015a; Rjeibi et al., 2017). On the contrary, all Tunisian dromedaries were tested negative (Belkahia et al., 2015b) (Fig. 1 and Table 1). The analysis of the Tunisian and Algerian *A. centrale* strains based on the *16S rRNA* gene sequence revealed nine different variants all grouped in a single cluster which is separated from *A. marginale* and *A. ovis* (Table 2, Belkahia et al., 2015a, 2017a; Rjeibi et al., 2017). Within this cluster, these North African strains have been classified into 3 sub-clusters suggesting an originated locally genetic diversity and/or multiple introductions of different strains of *A. centrale* in Tunisia and Algeria reported by Belkahia et al. (2015a, 2017a) and Rjeibi et al. (2017), respectively. Additionally, in Tunisia, the most frequent *A. centrale* variant reported by Belkahia et al. (2015a) has been shown to be identical to the vaccine strain from several sub-Saharan African and European countries (Bekker et al., 2002; Lew et al., 2003). Since attenuated live *A. centrale* vaccines were never used in Tunisia, it has been presumed that this strain was introduced by migratory birds and/or with cattle from countries where this vaccine has been licensed. In addition, other Tunisian strains closely related to a Japanese strain (Inokuma et al., 2001), which is relatively distant genetically to other *A. centrale* strains, have also been identified. However, further analysis of other genes will be required for a better characterization of these strains (Belkahia et al., 2015a).

2.3. Anaplasma bovis

Anaplasma bovis infects circulating monocytes (Sreekumar et al., 1996; Liu et al., 2012) and tissue macrophages of domestic and wild ruminants (Worthington and Bigalke, 2001). In general, it has been commonly reported in cattle, goats and wild deer (Liu et al., 2012; Ceci et al., 2014; Yang et al., 2014), and in other animal species including dogs (Sakamoto et al., 2010), rabbits (Goethert and Telford, 2003), cats (Sasaki et al., 2012) and small wild mammals (Masuzawa et al., 2014). In cattle, *A. bovis* infection has been reported as usually asymptomatic, but it can cause a variety of clinical signs, including reduced body weight, fever, anemia, depression, lymphadenopathy and rarely abortion, and death in some cases (Noaman and Shayan, 2010). The role of other animal species as natural carriers of *A. bovis* needs to be investigated.

To date in North Africa, the search of *A. bovis* infection has been performed only in Tunisia and Algeria. In fact, DNA of this pathogen was reported in all domestic ruminant species (cattle, sheep and goats) except camels (Belkahia et al., 2015a,b, 2017a,b; Ben Said et al., 2015b; Rjeibi et al., 2017) and the maximum of infection has been recorded in sheep (42.7%, Ben Said et al., 2015b) (Table 1 and Fig. 1). This *Anaplasma* species has also been molecularly characterized based on the *16S rRNA* gene (Table 2, Belkahia et al., 2015a, 2017a; Rjeibi et al., 2017). This analysis revealed a high identity between Tunisian variants isolated from goats, sheep and cattle (Belkahia et al., 2017a,b) and a high identity between an Algerian variant isolated from cattle and two genetic variants isolated from Japanese wild deer and South Korean *Haemaphysalis longicornis* ticks (Rjeibi et al., 2017).

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