EPIDEMIOLOGY AND DIAGNOSIS OF RICKETTSIOSES IN ANIMAL HOSTS AND TICK VECTORS

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Summary


Rickettsioses are emerging and reemerging vector-borne global diseases with zoonotic importance. The aim of this review is to give an overview on the epidemiology and diagnosis of rickettsial infection in animal hosts and tick vectors. In this review, some studies found out that companion animals such as dogs, horses and camels could serve as reservoirs or carriers of the disease. The dogs are mainly infested by Rhipicephalus sanguineus ticks that are the main vectors of Rickettsia conorii, while horses and camels can be infested by other ixodid tick genera such as Hyalomma and Amblyomma which are vectors of Rickettsia africæ beside other Rickettsia species in Africa including Egypt. The review also discusses the history of the disease, taxonomy, geographical distribution, prevalence, mode of infection and transmission, reservoirs or carriers, animal or tick susceptibility, pathogenesis, traditional and molecular diagnosis for rickettsiae and their tick vectors. Therefore, people dealing with animals such as farmers and veterinarians should be aware for the risk of exposures to ticks that may be infected with rickettsiae. Control tick programmes have to be applied from time to time to avoid any rickettsial infection to animals and humans.

Key words: animals, diagnosis, epidemiology, ixodid ticks, rickettsiae

INTRODUCTION

Rickettsioses are emerging and reemerging vector-borne global diseases with zoonotic importance (Parola et al., 2013). In Egypt, rickettsiae were detected in vectors, rodents, animals and human using different traditional and advanced techniques (Soliman et al., 1989; Corwin et al., 1992; Lange et al., 1992; Reynolds, 2004; Lofisi et al., 2006a,b; Socolovschi et al., 2010; Abdel-Shafy et al., 2012; Abdullah et al., 2016). The genus Rickettsia includes obligatory intracellular short Gram-negative bacillary bacteria. These microorganisms retain basic fuchsin when stained by Gimenez (Gimenez, 1964; Kang et al., 2014). The genus
Rickettsia is classified into spotted fever group (SFG) rickettsiae, typhus group (TG) rickettsiae, Rickettsia bellii group and Rickettsia candensis group (Merhej & Raoult, 2011). Rickettsiae particularly SFG are mainly transmitted through transstadial and transovarial routes by ticks (Raoult & Roux, 1997). Rickettsiae associated with ixodid (hard) ticks transmit the microorganisms to vertebrates through tick bites via their salivary secretions, or through faeces and blood transfusion (Socolovschi et al., 2009b).

In general, rickettsioses have low mortality but with high morbidity except some Rickettsia spp. which are characterised with high mortality in dogs and people as R. rickettsii. Four to ten days after tick biting, the clinical signs of rickettsioses appear. The typical symptoms are headache, fever, rash, muscle pain, local lymphadenopathy. A characteristic eschar (tache noire) takes place at the site of tick bite. The clinical signs of rickettsioses may differ from one rickettsial species to another. Common non-specific clinicopathological abnormalities due to rickettsioses include mild leukopenia, anaemia, and thrombocytopenia. Furthermore, hepatic, renal abnormalities, hypotension and hypoaalbuminemia may occur (Raoult & Roux, 1997; Parola et al., 2005; 2013).

Recently, the diagnosis of rickettsial infection depends on molecular tools that have facilitated the identification of new and previously recognised rickettsiae (Parola et al., 2013). The two major genes in SFG rickettsiae are gltA (citrate synthase) and OmpA (outer membrane protein A) (Roux et al., 1996; 1997, Fournier et al., 1998a; Mediannikov et al., 2004). These two genes have high variability between species of SFG rickettsiae (Roux et al., 1996; Guillemi et al., 2015).

An accurate identification of tick species is important to control any disease transmitted by ticks. Traditionally, taxonomic keys of tick species mainly depend on the morphological description of female and males adult by light microscope or scanning electron microscope. It is difficult to discriminate tick specimens by traditional techniques when they are fully engorged, physically damaged, in immature stages (Caporale et al., 1995; Guiglielmone et al., 2006) and at subspecies or at one group (Dantas-Torres et al., 2013; Gray et al., 2013). Recently, the molecular identification of ticks by DNA markers is essential. The DNA markers include nuclear (18S ribosomal RNA), mitochondrial (cytochrome oxidase subunit-1, 12S ribosomal RNA and 16S ribosomal RNA) genes and nuclear regulatory non-translated stretches (ribosomal internal transcribed spacer 2) (Chen et al., 2012; Nava et al., 2012; Lui et al., 2013; Lv et al., 2014a,b).

The aim of this review is to give an overview on the epidemiology and diagnosis of rickettsial infection especially in animal hosts and tick vectors. This review discusses some topics related to rickettsioses as history of the disease, taxonomy, geographical distribution, prevalence, reservoirs or carriers, tick or animal susceptibility, traditional diagnosis by stain and molecular diagnosis in animal hosts and tick vectors. Furthermore, the molecular identification of tick vectors was spotlighted in this review.

HISTORY OF RICKETTSIOSES

In 1899, tick-borne rickettsiosis was firstly described clinically as Rocky Mountain spotted fever (RMSF) by Maxey (1899). King (1906) and Ricketts (1906; 1909) reported that the wood tick...
**Dermacentor** has a role in the transmission of the causative agent of RMSF. Ricketts also demonstrated that the causative agent of RMSF can be transmitted from infected ticks to their progeny transovarially and that the organism can be isolated in guinea pigs. Moreover, Ricketts died of typhus and RMSF and the causative agent was subsequently named *Rickettsia rickettsii* (Ricketts, 1906; 1909). In 1933, dogs were reported to be susceptible to infection with *R. rickettsii* by Badger (1933). Also, Keenan et al. (1977a,b) induced experimentally infection in dogs with *R. rickettsii*. The first evidence of naturally occurring RMSF in dogs was reported by Lissman & Benach (1980).

In Tunisia, Conor & Bruch (1910) reported the first case of Mediterranean spotted fever (MSF). Thereafter, the disease was known as Boutonneuse Fever. In 1925, Marseille, the typical inoculation eschar was described at tick bite site (Olmer, 1925). Brumpt (1932) demonstrated the role of *Rh. sanguineus* tick in transmission of *Rickettsia conorii* (the causative agent of MSF).

For many decades, the rickettsiologists believed that the sole rickettsial agents were associated with specific sites (Raoult & Roux, 1997). In America, *R. rickettsii* was believed to be the only agent of SFG. In the same line, *R. conorii*, *Rickettsia sibirica*, *Rickettsia australis* was reported in Europe, (Africa or China) and Australia, respectively.

Until the 1990s, *R. conorii* was considered the only rickettsial agent in Africa (Raoult & Roux, 1997). In 1990, *Rickettsia africae* was isolated from the tick *Amblyomma hebraeum* in Zimbabwe by micro-immunofluorescence typing (Kelly & Mason, 1990; Kelly, 2001). Kelly and colleagues isolated *R. africae* by shell vial cell culture and named the disease African tick-bite fever (ATBF), as a newly recognised *Rickettsia* (Kelly et al., 1991). In Southern Africa, *A. hebraeum* was considered a vector of *R. africae* (Kelly et al., 1996). Furthermore, *R. conorii* and *R. africanae*, seven additional species of SFG rickettsiae were reported to be rickettsial pathogens in Africa (Letaief, 2006; Cazorla et al., 2008).

Another *Rickettsia* species were reported and isolated in Africa as *Rickettsia aeschlimannii*. In Morocco during the year 1992, *R. aeschlimannii* was firstly isolated from *Hyalomma marginatum* and in 1997 it was considered a new SFG *Rickettsia* (Beati et al., 1997). *Hyalomma* sp. were reported to be vectors and reservoir of *R. aeschlimannii* (Matsumoto et al., 2004). Moreover, the novel rickettsial agent, *R. massiliae* was isolated from *Rh. sanguineus* collected near Marseille, France (Beati & Raoult, 1993). In North Africa, *R. massiliae* was found in *Rh. sanguineus* and *Rh. bursa* ticks collected from Morocco (Sarih et al., 2008; Boudembough et al., 2009). This rickettsial species was also detected in *Rh. turanicus* and *Rh. sanguineus* ticks collected from Algeria (Bitam et al., 2006) and in *Rh. sanguineus* collected from Tunisia (Khrouf et al., 2014).

In Egypt, tick-borne rickettsial agents were reported serologically in animals and people (Botros et al., 1989; Soliman et al., 1989; Corwin et al., 1992; 1993; Reynolds, 2004). SFG of *Rickettsia* species were detected in *Hyalomma* species and *Rh. sanguineus* collected from Sinai using immunostaining method and PCR (Lange et al., 1992; Loftis et al., 2006a,b). Sokolovshi et al. (2010) reported *R. sibirica mongolitimonae* in a traveller from Egypt returned to France. Moreover, *R. africanae* was reported for the first time in
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The genus *Rickettsia* comprises obligatory intracellular short rods, cocci or thread-like Gram-negative bacillary bacteria. These microorganisms retained basic fuchsin when stained by Gimenez (Gimenez, 1964; Kang et al., 2014). This hemolymph staining technique was described in the mid 1950s (Gimenez, 1964). An extensive reorganisation was performed on the taxonomy of rickettsiae (Raoult & Roux, 1997; Hechemy et al., 2003). The order Rickettsiales is divided into two families – Anaplasmataceae and Rickettsiaceae. The sequences of five genes (*gltA, ompA, 16S rRNA, ompB*, and *sac 4 or gene D*) are recently used in classification of rickettsiae at the level of species, group and subspecies (Dumler et al., 2001; Fournier et al., 2003). The genus *Rickettsia* is divided into four groups; typhus (TG), spotted fever (SFG), *Rickettsia belli* and *Rickettsia candensis* (Fournier & Raoult, 2007; Merhej & Raoult, 2011). Moreover, Fournier and his colleagues mentioned that the most variable sequences at the level of rickettsial species or strain were intergenic spacers (Fournier et al., 2004).


EPIDEMIOLOGY OF RICKETTSIOSES

In general, the epidemiology of arthropod-borne diseases is affected by the disease triangle that includes three main factors: pathogen, host (vector, animal and human) and environment (Harrus & Baneth, 2005).

**Geographical distribution**

In general, rickettsioses are global emerging and reemerging vector-borne infectious diseases (Parola et al., 2005; 2013). Moreover, SFG rickettsioses are geographic disease because each tick species prefers specific optimal environmental conditions, biotopes and hosts (Parola & Raoult, 2001). Therefore, the distribution of ticks is restricted to a particular area that is considered risk area for the disease (Parola et al., 2005). Letaief (2006) affirms that the geographical distribution of rickettsiae is determined by the incidence of their arthropod hosts. The seasonal
Incidence of rickettsioses is parallel to the tick activity.

For example, MSF caused by *R. conorii* is endemic in Mediterranean areas. *Rhipicephalus sanguineus*, the main vector of *R. conorii*, could survive in warm climates and even cooler regions. It attacks dog kennels and humans that live in these climates. However, *R. conorii* had not been described in USA (Parola et al., 2005).

Various tick vectors were previously considered to be restricted to a particular geographical area. Recently, many SFG rickettsiae were recognised and detected on different continents (Parola et al., 2013). For example, *R. africae* was SFG restricted in central and southern Africa and its vector was *Amblyomma* spp. (Kelly et al., 1996; Macaluso et al., 2003; Socolovschi et al., 2007), but it was recently isolated from *Hyalomma* spp. in Egypt (Abdel-Shafy et al., 2012; Abdullah et al., 2016). In Brazil, Silva et al. (2018) recorded *Rickettsia amblyommatis* in the tick *Amblyomma pseudoconcolor*. In Panama, Martinez-Caballero et al. (2018) firstly detected *R. rickettsii* in *R. sanguineus*. In South America, three rickettsial strains, Atlantic rainforest, NOD, and Parvitarsum were isolated from *Amblyomma ovale*, *Amblyomma nodosum*, and *Amblyomma parvitarsum* ticks, respectively. These three strains are phylogenetically closely related to *Rickettsia parkeri*, *R. africae*, and *R. sibirica* (Neri-Bastos et al., 2018). In Bulgaria, Nader et al. (2018) detected by sequencing the presence of *Rickettsia monacensis*, *R. helvetica*, and *R. aeschlimannii* in ticks belong to the genera *Dermacentor*, *Hemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*. Furthermore, rickettsiae was determined in either animal hosts or tick vectors in many countries such as Malta (Torpiano & Pace, 2018), Argentina (Saracho-Bottero et al., 2018), Nigeria (Kamani et al., 2018), China (Han et al., 2018), Mongolia (von Fricken et al., 2018), Cameroon (Vanegas et al., 2018), Lebanon (Fernández de Mera et al., 2018), USA (Eremeeva et al., 2018), Russia (Igolkina et al., 2018) and Poland (Stańczak et al., 2018).

**Prevalence of rickettsioses**

In general, rickettsioses are characterised by high morbidity and low mortality rates except some *Rickettsia* spp. which induced high mortality in dogs and people as *R. rickettsii* in Brazil (Brazilian spotted fever) with challenging diagnosis due to its non-specific signs (Walker, 2002; Labruna et al., 2009). However, MSF had a global death rate of 3.6% (Kernif et al., 2012b). Fatality rates in dogs infected with rickettsiae in the USA were reported to be 3% (Greene, 1987) and 7% (Greene et al., 1985) while the incidence rate of ATBF was 1.9% in Norway and 25% in the Equator but may reach 74% of travelers’ cases in South Africa when occurring in clusters (Fournier et al., 1998b; Caruso et al., 2002; Jensenius et al., 2003).

Currently, limited information is available on the prevalence of rickettsioses in animal hosts. Dogs have been considered sentinels and sometimes horses were found in dog habitats (Campos et al., 2016). However, seroprevalence of *Rickettsia* spp. in dogs and horses in endemic region such as Juiz de Fora and Minas Gerais in Brazil was high; up to 68–81% (Vianna et al., 2008; Pacheco et al., 2011), while in non-endemic area in Brazil, the seroprevalence was less than 15% (Sangioni et al., 2005; Cunha et al., 2014; Silveira et al., 2015). In camels, Kamani et al. (2015) detected 18.8% *Rickettsia* spp. in camel blood samples.
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from Nigeria, Mentaberre et al. (2013) reported 83% Rickettsia spp. in camels by ELISA in Spain and Wernery et al. (2001) detected the rickettsiae in stained blood films in Dubai, UAE. In Lithuania, Mardosaitė-Busaitienė et al. (2018) stated that the prevalence of Rickettsia spp. in small mammals was 27.6%. In Germany, the prevalence of Rickettsia in rodents was determined by real-time PCR targeting gltA, ompB and ompAIV genes (Fischer et al. 2018). They found Rickettsia helvetica (90.9%), Rickettsia felis (7.8%) and Rickettsia raoultii (1.3%).

In ticks, the transstadial and transovarian system transmitted rickettsiae to vertebrate hosts during blood meal, which maintained bacteria in nature and made ticks simultaneously vectors and reservoirs (Raoult & Roux, 1997; Parola et al., 2005). Under field conditions, the rickettsial infection incidence tends to be lower than 1% because the rickettsiae can kill ticks (Niebylski et al., 1999; Levin et al., 2009; Socolovschi et al., 2009a). Therefore, the infection rate of Rickettsia spp. in Rh. sanguineus was reported to be 1.3–2.9% (Demma et al., 2005; Moraes-Filho et al., 2009). So, it is thought that vertebrates play a more dominant role as a reservoir and persistence of rickettsiae than ticks. In Brazil, Silva et al. (2018) found that 90.9% of the ticks Amblyomma pseudoconcolor collected from Euphractus sexcinctus were positive for infection with Rickettsia amblyommatis. In Bulgaria, the high prevalence of Rickettsia spp. (48.3%) was detected by using quantitative real-time PCR in ticks collected by flagging method (Nader et al., 2018). In Romania, Andersson et al. (2018) detected Rickettsia helvetica, R. raoultii, R. massilae, R. monacensis, R. slovaca and R. aeschlimanni in the ticks collected from domestic and wild animals. They found that the prevalence of rickettsial infection was 10.6% (87/824) of ticks.

Mode of infection and transmission

Rickettsioses are transmitted to vertebrate hosts by bite of infected ticks through salivary secretions and blood transfusion (Raoult & Roux, 1997). The salivary glands of ticks facilitate the feeding of ticks and act as vehicles, and propagation sites for rickettsiae (Santos et al., 2002; Socolovschi et al., 2009a). However, the infection by tick faeces is unknown. The faeces transmission proves the low infection of R. rickettsii in guinea pigs (Philip, 1959; Rehacek, 1965).

Hard ticks acquire rickettsiae through different sources. The initial tick infection with rickettsiae occurs by feeding of non-infected ticks on rickettsaemic hosts. Sufficient blood levels of rickettsiae in vertebrate hosts are required for infection (Rehacek, 1989). Also, ticks become infected with rickettsiae through transovarial transmission (Parola & Raoult, 2001; Anderson & Magnorelli, 2008; Socolovschi et al., 2009b). The other transmission route is the transstadial transmission in which rickettsiae transfer from stage to stage (Parola & Raoult, 2001). Sexual transmission is reported for acquiring rickettsiae by infected male ticks to non-infected females (Schriever & Azad, 1994). In addition, cofeeding method in which several ticks feed at closely situated bite sites in reported, which leads to direct rickettsiae transmission from infected to uninfected ticks (Philip, 1950).

Reservoirs and carriers

SFG rickettsiae circulate between wild vertebrates and arthropods vectors (Telford & Parola, 2007). As early as 1967, Burgdorfer and Varma stated that the most Ixodidae infected with SFG
rickettsiae were considered vectors and reservoirs. This attributes to the transstadial and trasovarial transmission of rickettsiae (Burgdorfer & Varma, 1967; Parola et al., 2005).

Dogs play an important role in maintaining rickettsial infection in the nature. In this way, the dogs obtain R. conorii infection from infected R. sanguineus and transmit rickettsiae to another uninfected tick (Levin et al., 2012). That is the first evidence that dogs can act as reservoir for rickettsiae (Piranda et al., 2011). Other authors suggested that capybaras and opossums as well as wild mammals and birds play an important role in amplifying and keeping of rickettsiae in nature (Horta et al., 2004; Souza et al., 2009; Keysary et al., 2011; Socolovschi et al., 2011; Movila et al., 2012; Ionita et al., 2016). Other studies suggested some other potential animal reservoirs of Rickettsia spp.; cattle and camels for R. africae (Reye et al., 2012), wild boars and domestic ruminants for R. slovaca (Ortuno et al., 2007; 2012) and sika deer for R. helvetica (Inokuma et al., 2008); however, additional studies are required to confirm animal reservoirs.

Animal susceptibility

Dogs and horses are important animals that have a role in the epidemiology of SFG rickettsioses. They act as keepers and amplifier hosts for rickettsiae due to their contacts to vegetations, ticks and other elements of the nature. Horses cover great areas and are able to spread the ticks infected with rickettsiae (Lemos et al., 1996; Sangioni et al., 2005; Freitas, 2007). Meanwhile, dogs are bringing infested ticks from outside to indoors and can transmit the rickettsial infection to humans (Medade & Newhouse, 1986).

Dogs can serve as a readily available source of nutrition for many blood-feeding arthropods and are frequently exposed to tick infestation (Otranto et al., 2010). They serve as indicator and epidemiological marker for the presence of vector-borne diseases such as rickettsioses (Tesouro et al., 1998; Ortuno et al., 2009). Dogs are human companions, extensively exposed to tick infestation, so they play a role as sentinels in an epidemiological approach of rickettsioses (Campos et al., 2016). Some cases of R. rickettsii were reported in dogs and their human owners in the USA (Paddock et al., 2002; Elchos & Goddard, 2003; Kidd et al., 2006) as well as in Brazil (Pinter et al., 2008; Piranda et al., 2011). Also, dogs are considered to be the sentinels of R. conorii infection (Parola et al., 2005; Ortuno et al., 2009).

Regarding the sex of animals, no sex predilection has been observed by Greene et al. (1985) and Gasser et al. (2001), while other reports demonstrated that males were more susceptible to R. conorii infection than females (Solano-Gallego et al., 2006a; 2008). Both male dogs and men are at risk for more severe illness with R. rickettsii and R. conorii (Parola et al., 2005; Greene & Breitschwerdt, 2006).

Moreover, Solano-Gallego et al. (2006b) recorded a significant difference between R. conorii infection rates in two dog breed groups, Yorkshire terrier and mixed breed. In contrast, no significant difference was reported in R. rickettsii positive samples from Yorkshire terrier dogs. However, the purebred dogs were more susceptible to R. rickettsii infection (Greene & Breitschwerdt, 2006).

Higher seroprevalence was reported in dogs highly exposed to R. sanguineus than in dogs living as pets or in kennels and subjected to tick control programmes.
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(Ortuno et al., 2009). Greene et al. (1985) found a predilection for dogs less than 2 years of age with respect to infection with R. rickettsii, whereas in another study, 93% of dogs infected with RMSF were older than 2 years (Gasser et al., 2001). Moreover, Cunha et al. (2014) observed that older animals were more reactive than younger animals, which may be attributed to the prolonged repeated exposure to ticks of older animals or low immunity. Therefore, there was a significant difference among age groups.

The participation of horses as reservoir of infection is arguable. Some literature sources reported high prevalence of rickettsiae in both horses and dogs (Lemos et al., 1996; Horta et al., 2004; Sangioni et al., 2005; Pinter et al., 2008) while Cunha et al. (2014) stated that horses were bad references for circulation of rickettsiae. Moreover, Sangioni et al. (2005) and Riveros-Pinilla et al. (2015) suggested that the presence of antibodies in horses is a decent marker of the spread of R. rickettsii in zones.

Riveros-Pinilla et al. (2015) reported the absence of statistical significant association between infection rate with Rickettsia spp. in horses and age, sex and breed, in line with Andersson (2013) who suggested that age, sex and breed in horses were not considered risk factors.

In camels, rickettsioses were reported in blood film staining by Wernery et al. (2001), while Wernery & Kaaden (2002) mentioned that the disease had not been reported in old world camels and these animal hosts had no role in the cycle of rickettsiae. However, some data reported the presence of SFG rickettsiae in some species of the genus Hyalomma collected from camels (Abdel-Shafy et al., 2012; Demoncheaux et al., 2012; Kernif et al., 2012a; Abdullah, 2017). Moreover, rickettsiae were detected in camels by ELISA (Mentaberre et al., 2013) and by PCR in Nigeria (Kamani et al., 2015). In general, camels come in contact with other animals at the livestock markets. This facilitates the transfer of ticks from one animal to another. On the other hand, Hyalomma spp. are able to attack humans (Mediannikov et al., 2010). This ability facilitates the transmission of Rickettsia spp. to humans who deal with camels.

Humans are only accidental or occasional hosts and rarely involved in subsequent transmission of rickettsiae, so they must be considered an end host that has no role in the maintenance of rickettsiae (Socolovschi et al., 2009b). Several reports stated the RMSF in humans with dog companions (Paddock et al., 2002; Elchos & Goddard, 2003).

**Tick susceptibility**

Rickettsia rickettsii is transmitted by several tick genera as Dermacentor spp. (King, 1906; Ricketts, 1906; 1909), and other ticks as Haemaphysalis leporispalustris, Amblyomma americanum, Ixodes dentatus and Rh. sanguineus (Parker et al., 1943; 1952). Rickettsia conorii was associated with one tick vector, Rh. sanguineus (Brumpt, 1932). Moreover, R. africae in South Africa was associated only with Amblyomma spp. (Parola & Raoult 2001; Parola et al., 2005), but now Hyalomma spp. can transmit R. africae in North Africa (Abdel-Shafy et al., 2012; Kernif et al., 2012a; Kleinerman et al., 2013; Abdullah, 2017). Also, Ogo et al. (2012) reported R. africae in Rh. sanguineus.

Lalzar et al. (2012) reported that the seasonal changes could influence the facultative relationship between Rickettsia and its tick vector. Moreover, the densities
of rickettsiae were equal through male and female ticks.

**Molecular identification of tick vectors**

Some morphological features are similar at both intra-species and inter-species level. This similarity limits the accuracy of morphological taxonomic key especially in *Rh. sanguineus* group (Walker et al., 2000; Guglielmone et al., 2014; Nava et al., 2015). However, molecular tools provide an accurate characteristics at the level of species and subspecies confirming morphological identification. Historically, the phylogeny of hard ticks using 12S rDNA sequences and morphological characters were studied by Beati & Keirans (2001). They concluded that the genus *Boophilus* was monophyletic and arose within the genus *Rhipicephalus*. Other studies agreed with this conclusion but using the sequences of CO1, ITS2 and 18S rRNA besides 12S rRNA (Murrell et al., 2001; Barker & Murrell, 2003; Murrell & Barker, 2003). In Egypt, the sequence and phylogenetic analyses of five DNA markers (18S rDNA, ITS2, 12S rDNA, CO1 and 16S rDNA) were established for molecular taxonomy of *Rh. sanguineus* and *H. dromedarii* (Abdullah et al., 2016). They confirmed the suitability of mitochondrial genes (12S rDNA, CO1 and 16S rDNA) for more accurate identification of tick species than the nuclear ones (18S rDNA and ITS2).

Over the last decade, many studies confirmed the suitability of DNA markers for rapid and reliable species identification of ticks (Latrofa et al., 2013). These molecular markers included nuclear i.e. 18S rRNA, mitochondrial i.e. 12S, 16S rRNA, and CO1 genes and nuclear regulatory non-translated stretches i.e. ITS2 (Chen et al., 2012; Nava et al., 2012; Lui et al., 2013; Lv et al., 2014a,b). The 18S rRNA was used for taxonomy at generic level (Dobson & Barker, 1999; Mans et al., 2011) whereas 16S rRNA, CO1, and ITS2 were the best markers at the tick species level (Mangold et al., 1998; Norris et al., 1999; Guglielmone et al., 2006; Lynn & Strüder-kypke, 2006; Chitimia et al., 2010; Song et al., 2011; Chen et al., 2012; Nava et al., 2012; Lui et al., 2013; Lv et al., 2014a,b). Furthermore, 12S rRNA was found suitable in determining the relationships between diverged branches in recent phylogenies of the tick taxonomy (Norris et al., 1999; Beati & Keirans, 2001; Lv et al., 2014b).

Several studies were applied for tick species identification using mitochondrial and nuclear markers which reported that the mitochondrial genome had strict maternal inheritance (Navajas & Fenton, 2000; Erster et al., 2013). On the other hand, a single pair of mitochondrial primers produced small-size PCR products from different species with fast evolution than nuclear ones (Shao & Barker, 2007). Moreover, the reference mitochondrial database is more available than nuclear marker and facilitates comparing the new sequences obtained from tick samples with that recorded before due to increased number of mitochondrial genes and genomes of different tick species (Beati & Keirans, 2001; Dergousoff & Chilton, 2007; Shao & Barker, 2007; Chitimia et al., 2010; Song et al., 2011).

**PATHOGENESIS OF RICKETTSIOSES**

Pathogenic SFG rickettsiae invaded animal hosts and humans through the bite of infected ticks into the endothelial cells of blood vessels (Raoult & Roux, 1997). Pathogenesis of rickettsiae was variable among different species according to the expression of particular rickettsial genes.
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(Parola et al., 2005). The OmpA gene in R. rickettsii helps the adhesion and entry of rickettsiae inside the endothelial cells (Li & Walker, 1998). Also, the OmpB played the same role in R. japonica (Uchiyama, 2003).

After internalisation and phagocytosis, rickettsiae lyse the phagosomal membrane to propagate in the cytoplasm and nucleus. Rickettsiae move between cells by actin mobilisation (Walker et al., 2003). The target cells of rickettsiae are endothelial cells and they spread in all organs including brain and lungs (Walker et al., 2003). Rickettsial pathogens are harboured in internal organs and tissues especially bone marrow, which is opposed to the infiltration of antibodies and antibiotics. Therefore, the persistence of rickettsiae in bone marrow may lead to relapse even with a prolonged treatment (Stiles, 2000; Levin et al., 2014).

Rickettsiae replicate in the vascular endothelium causing vasculitis, resulting in platelets and coagulation system activation, thrombosis, increased vascular permeability. Moreover, cytopathic effects and cellular activity of Rickettsia endotoxins triggered haemostatic disorders as thrombocytopenia and prolonged clotting time (Davidson et al., 1990).

The pathogenesis of R. rickettsii in dogs includes a combination of three mechanisms. The first is endothelial cell response to injury leading to promotion of a proinflammatory and thrombotic state. Second, microvascular thrombosis and endothelial injury cause oxidative stress with subsequent cell death. Finally, platelet homeostasis is further affected by immune-mediated platelet destruction (Silverman & Santucci, 1988; Eremeeva & Silverman, 1998).

Animal models (as guinea pigs) have been used to predict the pathogenicity of various rickettsiae but this technique turned out to be unreliable with them as some highly pathogenic strains of Rickettsia spp. produced only mild illness in guinea pigs. However, the determination of pathogenic role of tick-borne rickettsiae was dependent on isolation and detection of organism from host with signs of disease (Parola et al., 2005).

DIAGNOSIS OF RICKETTOSIES

The diagnosis of rickettsial infections is often difficult. In early stage of infection, the symptoms resemble several infectious diseases. A history of exposure to the tick vector is helpful but cannot be relied upon (Kernif et al., 2012b).

Clinical signs, haematological and biochemical changes

Dogs were reported to be infected with R. conorii (Estrada-Pena & Venzal Bianch, 2006; Solano-Gallego et al., 2006b; Levin et al., 2012) and R. rickettsii (Breitschwerdt et al., 1988; Gasser et al., 2001; Labruna et al., 2009). Only pain, erythema and oedema were observed in dogs experimentally infected with R. conorii (Kelly et al., 1992). Solano-Gallego et al. (2006b) observed febrile illness in dogs infected with R. conorii while other reports stated that no statistically significant differences existed between sick and clinically healthy dogs (Solano-Gallego et al., 2006b; Ortuno et al., 2009).

Canine rickettsioses caused by R. conorii were characterised by sub-clinical infection and less commonly acute diseases (Kelly et al., 1992). Acute clinical disease was observed in dogs infected with R. rickettsii (Solano-Gallego et al., 2006a, 2008). Therefore, the clinical signs observed in dogs experimentally infected...
with *R. rickettsii* included fever, lethargy, anorexia, bilateral ocular discharge, scleral congestion, conjunctival oedema, ocular and oral petechiae and tremors. Moreover, haematological and biochemical changes were in the form of thrombocytopenia, leukocytosis and anaemia (Piranda *et al.*, 2008; 2011; Levin *et al.*, 2014). In the advanced stage of *R. rickettsii* infection, dogs suffered from oedema in the extremities that may involve the lips, ears, penile sheath and scrotum (Greene & Breitschwerdt, 2006).

In canine RMSF, ocular manifestations are considered useful diagnostic signs (Davidson *et al.*, 1989). They reported that the ocular findings included bilateral conjunctival vascular injection, multifocal retinal haemorrhages, anterior uveitis and petechial haemorrhages in the iris stroma, whereas ocular haemorrhages were the most common ophthalmic sign of RMSF (Stiles, 2000). However, the most common cutaneous lesions in RMSF were oedema as well as petechial and ecchymotic haemorrhages and severe dermal necrosis in region in which oedema and haemorrhage were severe such as scrotum, pinnae and limbs (Weiser & Greene, 1989).

In a retrospective study on 30 dogs with RMSF, Gasser *et al.*, (2001) reported that the most common clinical signs were lethargy, anorexia, fever, vomiting, diarrhoea, ocular abnormalities, weight loss, lymph adenomegaly, oedema and ocular signs (sclera injection and retinal vasculitis), and less common clinical signs comprised arthropathic, cutaneous necrosis, petechiae, ecchymosis, myalgia and neurologic dysfunction including ataxia, hyperesthesia, vestibular signs and seizures (Low & Holm, 2005). In the same study Gasser *et al.* (2001) found that 85% of dogs infected with RMSF were thrombocytopenic and manifested other laboratory abnormalities as leukopaenia early in the course of disease followed by progressive leukocytosis, increased liver enzymes, coagulation abnormalities, anaemia, hypoalbuminaemia, hypercholesterolaemia, hyponatraemia and lymphocytic pleocytosis in cerebrospinal fluid. Moreover, Levin *et al.*, (2014) reported that RMSF infected dogs develop pronounced monocytosis and granulocytosis after five days from experimental inoculation. Meanwhile, Scorpio *et al.* (2008) stated that there were no specific haematological or biochemical differences between seronegative and seropositive dogs with respect to rickettsiae.

SFG rickettsioses were found to be fatal in some infected dogs and their owners as reported by Elchos & Goddard (2003). They reported two dogs suffering from seizure, petechiae of the gums and sclera, inability to stand, pale mucous membranes, fever and mild oedema of the face and forelimbs. In addition, laboratory abnormalities included leukocytosis, severe thrombocytopenia, hyponatraemia, hypercalcaemia, bilirubinaemia and hypoalbuminaemia, subsequently dogs died due to improper diagnosis and treatment.

In horses, no clinical signs were observed even with high titres of *R. rickettsii* and other rickettsiae and the clinical manifestation of the disease in horses is rare (Lemos *et al.*, 1996; Medeiros *et al.*, 2013). Riveros-Pinilla *et al.*, (2015) detected antibodies against *Rickettsia* spp. in apparently healthy horses.

Few data are available on SFG rickettsioses in camels and their clinical signs. Rickettsiae were detected serologically in camels by Mentaberre *et al.* (2013) and detected in stained blood film by Wernery *et al.* (2001). The latter authors reported some clinical features of
Rickettsiosis in camels as depression, lethargy, emaciation and recumbency and enlarged oedematous lymph nodes (Werney et al., 2001).

In general, many studies demonstrated the clinical signs of SFG rickettsioses in humans. The clinical symptoms began 4 to 10 days after tick biting and included fever, headache, muscle pain, rash, local lymphadenopathy and a characteristic inoculation eschar (tache noire) at the site of tick bite (Raoult & Roux, 1997; Watt & Parola, 2003; Parola et al., 2013). Common non-specific disorders associated with rickettsial infection included mild leukopenia, anaemia and thrombocytopenia, in addition to hepatic and renal disorders, hyponatraemia and hypoalbuminaemia (Raoult & Roux, 1997; Parola et al., 2005; 2013).

Blood film and hemolymph staining technique

In the blood of infected animal hosts, Rickettsia spp. circulated in low numbers even in the acute phase (Breitschwerdt et al., 1990; Parola, 2005). Blood film stained with a specific rickettsia stain (Gimenez stain) showed red cocci, rods or thread-like rickettsiae inside blood cells, as rickettsiae are compulsory intracellular short Gram-negative bacillary bacteria that held basic fuchsin (Gimenez, 1964). In ixodid ticks, rickettsiae circulated in the haemolymph of infected ticks (Santos et al., 2002). However, haemolymph staining technique by Gimenez was applied on haemolymph of ticks, keeping ticks undamaged for further identification and rickettsial isolation (Gimenez, 1964; Burgdorfer, 1970).

Molecular diagnosis of rickettsiae in animal hosts

The diagnosis of rickettsioses is a challenge because of non-specific clinical signs and laboratory abnormalities or subclinical infection (Gasser et al., 2001; Parola et al., 2005; 2013). Serological methods – microimmunofluorescence (MIF) and western blotting (WB) – were frequently used for diagnosis of SFG rickettsioses, but there are drawbacks of serological methods such as negative serological titres, antigenic cross-reactions among SFG rickettsiae (Breitschwerdt et al., 1990; Parola et al., 2005). Therefore, serological diagnosis of rickettsioses is adequate but insufficient for accurate identification of the causative agent (Parola et al., 2005; 2013). In addition, cell culture can detect, isolate and describe new species of rickettsiae but it is still difficult to be performed and few reference labs can apply it (Parola et al., 2005).

To avoid delay in diagnosis of rickettsioses, molecular techniques (including PCR and sequencing) were applied to allow more accurate and rapid detection and identification of rickettsiae with improved sensitivity and specificity of the diagnosis (Parola et al., 2013; Guilmeli et al., 2015). Primers were used in amplifying sequences of several genes including OmpA, OmpB, gltA and gene D (Roux et al., 1997; Fournier et al., 1998a; Sekeyova et al., 2001; Fournier et al., 2003; Brouqui et al., 2004). The gltA gene was less conserved in SFG rickettsiae, so it had a high discrimination power in Rickettsia spp. (Roux et al., 1997). Regnery et al., (1991) applied amplification of two gltA fragments to improve the species recognition. On the other hand, Roux et al. (1997) suggested that OmpA gene was specific for SFG rickettsiae. In another study the whole
OmpA gene was amplified and confirmed higher intra-SFG variability than that in gltA gene (Fournier et al., 1998a). Moreover, intergenic spacers (mppA, dksA and rpmE) were characterised by high recognition, reproducibility, interpretation effortlessness and simplicity of joining of the information into open database (Parola et al., 2005). These advantages of intergenic spacers were attributed to the variability of these spacers more than conserved, split and remnant genes (Fournier et al., 2004).

In dogs, few molecular surveys were applied for canine SFG rickettsioses (Estrada-Peña & Venzal Bianch, 2006; Solano-Gallego et al., 2006a). Levin et al. (2012) examined dogs by PCR and found that the great part of positive blood samples was recorded during the fever time and these results were reported also by Piranda et al. (2008). Solano-Gallego et al. (2008) reported that the percentage of rickettsial infection in the blood of sick dogs was 1.5% vs 14% in other studies (Estrada-Peña & Venzal Bianch, 2006; Torina et al., 2007). Also, Kamani et al. (2013) detected DNA of Rickettsia spp. in dog blood samples (8.8%) and in their ticks (10.5%) in Nigeria.

To our knowledge, there are no studies detecting infection of rickettsiae in horses by molecular methods (PCR). Most of the prevalence surveys were done by using immunofluorescence for the detection of antibodies against different Rickettsia species in sera of horses (Lemos et al., 1996; Horta et al., 2004; Medeiros et al., 2013; Alves et al., 2014; Riveros-Pinilla et al., 2015).

In camels, Kamani et al. (2015) identified gltA fragment (133 bp) of Rickettsia spp. in 18.8% of blood samples collected from camels in Nigeria. Wernery et al. (2001) demonstrated rickettsiae in blood films of camels using staining technique. Other studies in camels reported in the Canary Islands, Spain (Mentaberre et al., 2013) affirmed that 83% of the examined camels were infected with Rickettsia spp. using ELISA. In Colombia, Santodomingo et al. (2018) detected R. monocacensis and R. bellii in reptiles by using PCR and sequencing of the gltA, 16S rRNA, and sca1 genes.

In Egypt, there are few studies on animal hosts or humans, except the sero-survey of R. conorii infection which indicated that humans were exposed to SFG rickettsiae (Botros et al., 1989; Corwin et al., 1993). Moreover, Socolovschi et al. (2010) reported R. siberica mongolitimonae in a traveller from Egypt returned to France. Abdullah (2017) revealed that the infection rates of rickettsiae were 18%, 72% and 41% in dogs, horses, and camels, respectively by using PCR for amplification of OmpA and gltA genes. The author added that the phylogenetic analyses based on clustal omega suggested that detected rickettsiae sequences were R. africae-like in camels and dogs, and the first record of R. massiliiae-like in dogs.

Molecular diagnosis of rickettsiae in tick vectors

Application of molecular tools for the detection of rickettsial infection in ixodid ticks was used as epidemiological tool (Parola & Raoult, 2001). Many studies all over the world were carried out for detection and identification of SFG rickettsiae on tick vectors by molecular methods (Guillemi et al., 2015).

In North Africa, the most frequent circulating ticks were Rh. sanguineus and Hyalomma spp. (Bouattour, 2002). Rickettsia conorii was identified in Rh. sanguineus ticks from Algeria (Bitam et
al., 2006; Bessas et al., 2016), Tunisia (Sfar et al. 2009) and Morocco (Boudebouch et al., 2009). Moreover, R. aeschlimannii was first detected in H. marginatum in Morocco (Beati et al., 1997) and then detected by molecular tools in Algeria (Bitam et al., 2006), Morocco (Sarih et al., 2008) and Egypt (Abdel-Shafy et al., 2012). Rickettsia aeschlimannii was also identified in other Hyalomma spp. including H. dromedarii, H. impeltatum, H. rufipes, H. truncatum, H. aegyptium and H. excavatum collected from camel and cow hosts in Egypt, Sudan, Algeria and Tunisia (Abdel-Shafy et al., 2012; Demoncheaux et al., 2012; Djerbouh et al., 2012; Kernif et al., 2012a; Leulmi et al., 2016). Recently, R. africae was detected in H. dromedarii in infested camels in Algeria (Kernif et al., 2012a) and Egypt (Abdel-Shafy et al., 2012). R. massiliense was detected by PCR in Rh. sanguineus from Morocco (Sarih et al., 2008; Boudebouch et al., 2009), Algeria (Bitam et al., 2006; Khalidi et al., 2012; Bessas et al., 2016; Leulmi et al., 2016) and Tunisia (Khrouf et al., 2014).

In Egypt, SFG rickettsiae were detected in Rh. sanguineus and Hyalomma species from Sinai (Lange et al., 1992). Loftis et al. (2006a,b) detected R. aeschlimannii in Hyalomma spp. Recent studies reported for the first time R. africæ and R. aeschlimannii in H. dromedarii, H. impeltatum and H. marginatum collected from camels in Sinai (Abdel-Shafy et al., 2012; Abdulllah et al., 2016). The phylogenetic analyses of partial sequences of the two genes (OmpA and gltA) and sequences of three intergenic spacers (mppA, dksA and rpmE) of Rickettsia spp. in H. marginatum collected from a camel revealed a novel strain of R. africæ (Abdullah, 2017).

In Brazil, Moerbeck et al. (2018) isolated Rickettsia sp. from the tick Amblyomma nodosum. They found that Rickettsia sp. was close to Rickettsia sp. strain NOD, with 99.9%, 100.0%, and 99.8% similarity for gltA, htrA, and ompA genes, respectively. In Australia, Chalada et al. (2018) used PCR and sequencing for molecular survey of Rickettsia spp in midguts of soft and hard ticks. They found Rickettsia gravesii, Rickettsia felis, and other Rickettsia spp. In Lithuania, Rickettsia helvetica was first detected in small mammals by sequencing of gltA gene and the 17 kDa protein coding gene (Mardosaitė-Busaitienė et al., 2018). Kissenkötter et al. (2018) developed a rapid method for detecting Rickettsia spp. They targeted the 23S and 16S rRNA genes to develop a recombinase polymerase amplification assay that required between seven to ten minutes to amplify and detect one or ten DNA reactions.

CONCLUSION

This review provides valuable information on all aspects of rickettsiae in animal hosts and their tick vectors. Some studies found that animals accompanying humans such as dogs, horses and camels could serve as reservoirs or carriers of the disease. The dogs are mainly infested by Rh. sanguineus that is the main vector of R. conorii, while horses and camels can be infested by other ixodid tick genera such as Hyalomma, Amblyomma, vectors of R. africæ besides other Rickettsia spp. in Africa including Egypt. Therefore, people dealing with animals such as farmers and veterinarians should pay attention to ticks exposure that may be infected with rickettsiae. Tick control programmes have to be occasionally applied to avoid rickettsial infections to humans. Further
research studies are needed on a global scale to determine the biodiversity of Rickettsia spp. in domestic or wild animals and to detect their role in the distribution of rickettsioses. Climatic changes and animal movements between countries are the main challenges in diagnosis of rickettsiae. Therefore, it should investigate the animals and their tick vectors from time to time to monitor the epidemiology of the disease. The quick diagnosis helps appropriate decision to control the pathogens by treatment of infected animals or control of tick vectors.

REFERENCES


Abdullah, H. H. A. M., 2017. Some epidemiological studies and molecular characterization of rickettsiae infecting some animals in Egypt. PhD Thesis, Faculty of Veterinary Medicine, Cairo University, Egypt.


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sites in Algiers, Algeria. Comparative Immunology, Microbiology and Infectious Diseases, 45, 23–28.


Australia. Vector-Borne and Zoonotic Diseases, 18, 151–163.


Dobson, S. J. & S. C. Barker, 1999. Phylogeny of the hard ticks (Ixodidae) inferred from 18S rRNA indicates that the genus Apo-
tion of genera in the family's Rickettsiaceae and Anaplasmataceae in the
order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new
species combinations and designation of Ehrlichia equi and 'HGE agent' as subject


type in Haemaphysalis leporispalustris from California, USA. Ticks and Tick-borne Diseases, in Press, https://www.sciencedirect.com/sci
cence/article/pii/S1877959X17301747.


Fernández de Mera, I. G., V. Blanda, A. Tori
na, M. F. Dabaja, A. El Romeh, A. Cabe
zas-Cruz & J. de la Fuente, 2018. Identifi
cation and molecular characterization of spotted fever group rickettsiae in ticks collected from farm ruminants in Lebanon. Ticks and Tick-borne Diseases, 9, 104–108.


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Khalidi, M., C. Socolovschi, M. Benyettou, G. Barech, M. Biche, T. Kernif, D. Raoul &


Lynn, D. H. & M. C. Strüder-Kypke, 2006. Species of tetrahymena identical by small subunit rRNA gene sequences are discriminated by mitochondrial cytochrome c oxi-


Epidemiology and diagnosis of rickettsioses in animal hosts and tick vectors


Pinter, A., C. M. Horta, C. R. Pacheco, J. Moraes-Filho & B. M. Labrune, 2008. Serosurvey of *Rickettsia* spp. in dogs and humans from an endemic area for Brazil-
ian spotted fever in the State of Sao Paulo, Brazil. Cadernos de Saude Publica, 24, 247–252.


Ricketts, H. T., 1906. The transmission of Rocky Mountain spotted fever by the bite of the wood tick (Dermacentor occiden-


Ricketts, H. T., 1909. Some aspects of Rocky Mountain spotted fever as shown by recent investigations. Medical Record, 76, 843–855.


Epidemiology and diagnosis of rickettsioses in animal hosts and tick vectors


Solano-Gallego, L., L. Kidd, M. Trotta, M. Di Marco, M. Califin, T. Furlanello & E. Breitschwerdt, 2006b. Feverile illness associated with *Rickettsia conorii* infection in...


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