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Rickettsiales AND *Coxiella burnetii* INFECTIONS IN CAMELIDS: A REVIEW

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Overview

The members of the *Rickettsiales* Order are very small, non-motile, pleomorphic, obligate intracellular Gram-negative bacteria. They are coccobacilli or short rods, which are visible under light microscope best at 100 x oil magnification. *Rickettsiales* and *Coxiella* stain poorly with Gram but better with Giemsa and Romanowsky stains. Most of these bacteria do not grow on inert media. They require living cells for their replication and are normally cultured in tissue cultures (Munderloh *et al*, 2003), preferable tick cell cultures or in yolk sac of embryonated hen eggs (Passos, 2012).

The genus includes many species also associated with human disease, including those in the spotted fever and typhus group. The *Rickettsiae* that are pathogens to human beings are subdivided into three major groups based on clinical characteristics of the disease:

Spotted fever group with 8 species

Typhus group with 3 species

Scrub typhus group with 3 species

Rocky Mountain spotted fever caused by *Rickettsia rickettsii* for example is common in Mexico and North and South America and is transmitted by rodents, dog ticks like *Dermacentor* and *Amblyomma* species. In human beings the disease is characterised by fever, muscle pain, severe headache and occasionally by a myocarditis (Markey *et al*, 2013).

The classification of this group of bacteria is complex and complicated and not finalised, yet. For example, several species in the *Anaplasmataceae* family have been redesigned, as they previously included haemotrophic bacteria, which are now confirmed to be closely related to *Mycoplasma* as they also lack a cell wall.

The *Rickettsiales* Order comprises of two families of veterinary significance which are *Rickettsiaceae* and *Anaplasmataceae* (Markey *et al*, 2013). The family *Rickettsiaceae* possesses a cell wall, but members of the *Anaplamataceae* family lack a peptito glycan layer.

Significant re-classification of the Order has occurred several times over the years, which are mainly based on DNA sequencing in particular 16S and 23S-r RNA gene sequence comparisons. The classification is not yet complete.

The source of rickettsia taxonomy can be found in the latest (2004) edition of the Bergey's Manual of Systematic Bacteriology or under Schoch *et al* (2020), NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database Oxford 2020: baaa062. Pub Med: 32761142 PMC: PMC 7408187. This database gives an overview of *Rickettsiales* currently known. Most of them are either unclassified, uncultured or 'candidates' waiting for their classification; in total more 100 different species. However, the newest classification of *Rickettsiales* comprises the family *Rickettsiaceae* into two genera: Rickettsia and Orienta; both with no veterinary importance, but responsible for zoonotic diseases of human beings; while the family *Anaplasmataceae* have five genera: *Anaplasma, Ehrlichia, Neorickettsia, Aegyptianella* and *Wolbachia*.

Only few species of the *Anaplasmataceae* are pathogens of veterinary significance which are listed in Table 1.

As can be seen from this Table, none of them is mentioned to produce disease in camelids.

Coxiella burnetii, the cause of Q fever, is now closely related to *Legionella* species and *Franciscella tularensis* and is therefore dealt here in a separate section.

Natural Habitat and Pathogenesis

Members of the *Rickettsiales* are bacteria of arthopods which are replicating in the gut cells before spreading to other organs, such as salivary glands and ovaries. The requirement for an invertebrate vector, distinguishes these microorganisms from other bacterial species. This is unique. Infection

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Table 1. Rickettsial pathogens of veterinary significance according to Markey *et al* (2013).

Pathogen	Host	Vector	Country	Disease
Rickettsia rickettsii	Humans, dogs	Dermacentor species	Western hemisphere	Rocky Mountain spotted fever
Anaplasma marginale	Ruminants	Hard ticks	Tropics, sub-tropics	Gall sickness
A. ovis	Small ruminants	Hard ticks	Tropics, sub-tropics	Anaplasmosis
A. bovis	Cattle	Hyalomma	Africa, South America, Middle East, Asia	Bovine ehrlichiosis
A.platys	Canine	Ticks	America, Middle East, Mediterranean	Thrombocytopenia
A.phagocytophilum	Ruminants, horse, human beings	Ixodes	Worldwide	Tick-borne fever, equine and human granulocytic ehrlichiosis
Ehrlichia canis	Canine	Rhipicephalus	Tropics, subtropics	Canine monocytic ehrlichiosis
E. ewingii	Canine, human	Amblyomma	USA	Canine granulocytic ehrlichiosis
E. ovina	Ovine	Ticks	Africa, Asia, Middle East	Ovine ehrlichiosis
E. ondiri	Cattle	Ticks	East African highlands	Bovine petechial fever
Neorickettsia helminthoeca	Canine, bears	Salmonid fish ingestion	West Coast North America	Salmon poisoning disease
N. elokominica	Canine, bears, raccoons	Salmonid fish ingestion	North America	Salmon fever
N. risticii	Horse	Ingestion of aquatic insects	USA, Europe	Potomac horse fever
Aegyptianella pullorum	Birds	Argus species	Africa, Asia, Mediterranean	Aegyptianellosis

typically occurs as a result of a bite of an infected arthropod, mainly ticks. The pathogenesis varies widely with each species and a number of species persist in the host in a latent form. Identification of these microorganisms is not easy and is usually based on animal species infected, tick identification, clinical signs, demonstration of the bacteria in specimens, mainly blood, specific serological tests and PCRs. When the isolate has been obtained, sequence analysis of the genes should follow.

Rickettsiales in Camelids

Over the last decades several scientific papers have been published on tick-borne pathogens in camelids, either diagnosed during serological surveys or by molecular biological tools, especially PCRs using different primers. No publications were found describing culture methods in connection with this bacterial group. Some of the most important papers on rickettsial infections in camelids from different countries are found in Table 2. They also include *Rickettsiales* diagnosed in camel ticks. All investigations are so far snapshots and not long term studies. Additionally, with very few exceptions, most of these tests described in these publications have not been evaluated for use in camelids and all positive results were more or less from healthy camelids, showing no signs of illness with very few exceptions. Evaluation of serological tests is a prerequisite for a proper diagnosis as was recently shown by Soellner *et al* (2018), who evaluated many serological tests for the diagnosis of brucellosis in experimentally infected dromedaries. Parvizi *et al* (2020) evaluated a competitive ELISA for screening anaplasmosis, better *Anaplasma* infections, in camel populations in Egypt. Additionally, interpretation of results, where only staining methods were performed for the diagnosis of rickettsial infections, should be dealt with caution (Schuster *et al*, 2021), as it is often very difficult, if not impossible to diagnose intraplasmatic *Rickettsiae* correctly in blood smears. Some authors also exaggerate the effect of rickettsial infections in camels as causing significant losses in this species (Parvizi *et al*, 2020) or naming them "camel haemopathogens" (Kidambasi *et al*, 2020). So far only minor disease if any has been described in camelids and therefore one should use the word rickettsial infection instead of Rickettsiosis. It is also worthwhile mentioning, that *Anaplasma* species identified by PCR are named "*Candidatus Anaplasma*" (Lbacha *et al*, 2017), but other researchers are more confident that they have detected a new species that they named *Anaplasma camelii* without giving proper details. Some of these *Anaplasma candidatus* resemble

Table 2. Details of Rickettsiales species found in camelids and their ticks.

Rickettsiales Infection species	Authors	Test Kits	Results	Disease	Country
A. marginale, bovis, centrale	Wernery *et al* (2007)	cELISA VMRD, France	Blood 0.5% (5/1119)	None	UAE
A. marginale	Wernery *et al* (2014b)	PCR	Blood 0.0% (0/55)	None	UAE
A. marginale	Parvizi *et al* (2020)	cELISA PCR	Blood 1.6% (7/437) 1.6%	None	Egypt
Ca. A. camelii	Lbacha *et al* (2017)	PCR Gene: groEL	Blood 39.6% (42/106)	None	Morocco
Ca. E. regneryi *Ca. A. camelii* *C. burnetii* *Ca. E. regneryi* *Ca. A. camelii* *C. burnetii* *E. chaffeensis* *R. africae* *R. aeschlimannii*	Getange *et al* (2021)	PCR (DNA detection) PCR	Blood/ Ticks 80.1% (240/296) Camel Ticks *Hyalomma* *Amblyomma* *Rhipicephalus*	None	Kenya
A. platys	Li *et al* (2015)	PCR	Ticks (*Rhipicephalus sanguineus*) 7.2 % (20/279)	None	China
Ca. A. camelii	Kidambasi *et al* (2020)	PCR	Blood/ Ticks 68.67% (172/249) Camel Ticks *Hippobosca camelina*	None	Kenya
A. platys	Rassouli *et al* (2020)	PCR	Blood 3.3 % (2/60)	None	Iran
Ca. A. camelii	Sharifiyazdi *et al* (2017)	PCR	Blood 6.0 % (6/100)	None	Iran
E. ruminantium *E. canis* *Ca. E. regneryi*	Younan *et al* (2021)	PCR	Blood 2 camels	Heartwater-like disease	Kenya
A. phagocytophilum	Bahrami *et al* (2018)	PCR	Blood 34.2% (71/207)	None (subclinical?)	Iran
R. aeschlimannii *R. africae*	Kleinerman *et al* (2013)	PCR	Ticks 4.9% (3/148)	None	Israel
A. platys *A. canis*	Bastos *et al* (2015)	PCR	Blood 30.0% (30/100)	None	Saudi Arabia
A. phagocytophilum *A. marginale* *A. ovis* *Ca. A. camelii* *A. platys*	Azmat *et al* (2018)	PCR	Blood 13.3% (45/100)	Decreased white blood cell count	Pakistan
R. aeschlimannii *R. monacensis* *R. helvetica* *R. africae*	Selmi *et al* (2019)	Omp PCR	Blood 2.7% (8/293) *Hyalomma impeltatum* (10.4%) *H. dromedarii* (8.0%)	Not mentioned	Tunesia
A. platys	Belkahia *et al* (2015)	qPCR	Blood 17.7 % (40/226)	None	Tunesia

Abbreviations : A. = Anaplasma Ca. = Candidatus E. = Ehrlichia R. = Rickettsia C. = Coxiella H. = Hyalomma

Anaplasma platys and may not be a new species until proven. The high prevalence of *"Ca. A. camelii"* in healthy camels especially in Kenya seems to be an indication that the bacterium is either subclinical or non-pathogenic (Getange *et al*, 2021).

Candidatus (C.) *Anaplasma (A.) camelii* can be transmitted not only by ticks but also by the camel specific ked *Hippobosca camelina* as described by Bargul *et al* (2021). The authors also reported that the haematophagus ked transmit these bacteria to mice and rabbits via blood feeding. Sudan *et al* (2014) successfully treated subclinical anaplasmosis (*A. marginale*) in one dromedary camel in India showing anaemia and depression with a combination of different drugs.

In 2016, Younan *et al* (2021) described a heart water-like disease in Kenya but also in other countries (Onyiche *et al,* 2020; Alshahrani *et al,* 2020) which had killed 2000 adult animals. Gross pathology

showed pulmonary oedema, hydrothorax and hydropericardium. In the blood from two sick dromedaries, Ehrlichia species were identified by PCR resembling *E. ruminantium*, *E. canis* and "Candidatus *E. regneryi*". It was not clear, if any of these species were involved in this outbreak. Infection rates of *E. ruminantium* between 5.2% and 12.4% were reported by Getange *et al* (2021) in Kenya. These camels did not show any signs of heart water.

Anaplasmataceae were also reported in South American camelids. A llama suffered from granulocytic anaplasmosis and a strain was sequenced resembling *A. phagocytophilum* (Wernery *et al*, 2014b; Barlough *et al*, 1997). It has also to be stressed that special *Rickettsiae* species are only found in special ticks.

To overcome the uncertainity of a *Rickettsiae* infection in camelids, experimental infections are necessary to investigate, if this bacteria group is pathogenic to camels. This is, however a challenge, as many different rickettsial species have been described to occur in dromedaries, the most important ones are summarised in Table 2 with details of authors, test kits used, results, country of origin and disease details. The findings in Table 2 include also details about camel tick species and *Rickettsiae* species found in them.

Q Fever

Coxiellosis is caused by a Gram negative coccobacillus *Coxiella burnetii*, which does not belong anymore to the *Rickettsiales*, as phylogenetic analyses showed, that *C. burnetii* is more closely related to *Legionella* and *Franciscella* than to *Rickettsia* genus. This microorganism resides and replicates in its host's monocytes and macrophages. Two forms exist, the large cell variant is a vegetative form found in infected cells and the small cell variant is the extracellular infectious form shed in urine, milk and faeces. It is also found in very high concentrations in placental tissue and amniotic fluid like *Brucella* organisms. The disease is enzootic in most areas, where cattle, sheep and goats are kept; it is also a zoonotic disease and is frequently diagnosed in human beings, who have occupational contact with risk animal species like goats. A detailed overview of Q fever in dromedaries is presented in the OIE book compiled by Wernery *et al* (2014a).

So far no disease has been attributed to Q fever in camelids, but many serological investigations have been performed, most of them with serological prevalences between 2 and 80%.

Although a high prevalences has been reported from some African countries, the serological incidences in human beings, for example in Chad, were very low. However, antibodies against *C. burnetii* have been found in high numbers of livestock handlers in association with small ruminants (Getange *et al*, 2021). Belkahia *et al* (2020) found a serological prevalence of 75.5% in Algerian camels, but all 184 blood samples were negative in the PCR. However, five ticks from these dromedaries were PCR *C. burnetii* positive. Wernery (2011) reported that 45 raw camel milk samples originating from serologically positive dairy camels, were all Q fever negative using PCR technology. It is also worthwhile mentioning, that in this camel dairy farm, no Q fever abortions were reported. This is contrary to Q fever infected small ruminants.

Further studies are needed to better understand the role of camels in the epidemiology of Q fever and especially if they are or their products possess a zoonotic risk.

Resumé

Rickettsial bacteria, especially *Anaplasma* species have been found in dromedary and Bactrian camels either in their blood or in ectoparasites attached to their skin by molecular biological techniques by many researchers. Only very few serological investigations were carried out and no bacterial culture methods. These microorganisms were detected in healthy camels indicating the presence of asymptomatic carrier states. This comes as no surprise as camels are regularly infected by many different tick species, even sometimes covered by them without showing any signs of illness.

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