



Letters to the Editor

***Ehrlichia minasensis*, an old demon with a new name**

Dear Editor,

Since the first report of a novel *Ehrlichia* infecting cattle in British Columbia, Canada (Gajadhar et al., 2010), we have followed with great interest the epidemiology of these bacteria. Then, in 2012, this *Ehrlichia* was reported in free-ranging mule deer *Odocoileus hemionus* in the same region of Canada and was provisionally designated *Ehrlichia* sp. BOV2010. (Lobanov et al., 2012). Later that year, an *Ehrlichia* was isolated from the haemolymph of engorged *Rhipicephalus microplus* female ticks from Minas Gerais, Brazil, and based in 16SrRNA, the *Ehrlichia* from British Columbia and Minas Gerais were found to be the same microorganism (Cabezas-Cruz et al., 2012; Cabezas-Cruz et al., 2016). Further molecular analysis including the bacterial genes *groEL*, *dsb*, *gltA*, *trp36* (Cabezas-Cruz et al., 2012, 2014) and full genome sequencing (Cabezas-Cruz et al., 2015), allowed us concluding that this was a previously uncharacterized α -Proteobacteria of the family Anaplasmataceae, genus *Ehrlichia* and was officially named *Ehrlichia minasensis* (Cabezas-Cruz et al., 2016). The continuous propagation of *E. minasensis* was achieved for more than 1 year in IDE8 tick cells and canine DH82 cells (Zweygarth et al., 2013) and the specimen is available in the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ) under the accession = DSM 100393^T (Cabezas-Cruz et al., 2016). Morphological characterization of *E. minasensis* using electron microscopy revealed membrane-bound vacuoles in the cytoplasm of IDE8 cells. Vacuoles contained round- or oval-shaped bacteria of 0.4–1.5 μ m diameter. Reticulated and electron-dense forms were also observed. The morphology of *E. minasensis* resembled that of *Ehrlichia canis* and *Ehrlichia chaffeensis* strains (Cabezas-Cruz et al., 2013).

Apart from *E. minasensis*, *E. ruminantium* is the only other species of *Ehrlichia* known to naturally infect cattle. It is worth mentioning that an earlier report described an agent named *Ehrlichia bovis* in the monocytes of bovines in Rio de Janeiro and Minas Gerais, Brazil (Massard and Massard, 1982). However, in 2001, using molecular taxonomy, *E. bovis* was reclassified as *Anaplasma bovis* (Dumler et al., 2001).

Despite having the same host specificity, *E. minasensis* and *E. ruminantium* are in the furthest extremes in the phylogeny of the genus *Ehrlichia* (Cabezas-Cruz et al., 2016). *E. minasensis* is closely related to the monocytotropic pathogen *E. canis* and molecular evolution analysis suggested that *E. minasensis* originated from highly variable strains of *E. canis* (Cabezas-Cruz et al., 2014). In 2014, *E. minasensis* was found to naturally infect cattle in Brazil and to cause clinical ehrlichiosis in an experimentally infected calf in which *E. minasensis* morulae were observed in peripheral blood monocytes (Aguilar et al., 2014). The clinical symptoms caused by *E. minasensis* infection in bovines included fever, depression, lethargy and thrombocytopenia (Aguilar et al., 2014).

The distribution of *E. minasensis* is not restricted to the American continent as previously thought (Gajadhar et al., 2010; Cabezas-Cruz et al., 2012; Aguilar et al., 2014), and these bacteria were reported recently in Corsica, France (Cicculli et al., 2019), Pakistan (Rehman et al.,

2019), Ethiopia (Hailemariam et al., 2017), South Africa (Iweriebor et al., 2017) and Israel (Thomson et al., 2018). *E. minasensis* infects not only bovines (Gajadhar et al., 2010; Aguilar et al., 2014; Hailemariam et al., 2017) and cervids (Lobanov et al., 2012), but also dogs (Thomson et al., 2018). Although vector competence remains to be tested, *E. minasensis* has been identified in several tick species including *R. microplus* (Cabezas-Cruz et al., 2012; Carvalho et al., 2016), *Rhipicephalus appendiculatus* (Iweriebor et al., 2017), *Hyalomma marginatum* (Cicculli et al., 2019) and *Hyalomma anatolicum* (Rehman et al., 2019), and so far it has not been detected in *Amblyomma sculptum* (Carvalho et al., 2016) *Rhipicephalus evertsi evertsi*, *Rhipicephalus sanguineus* s.l., *Amblyomma hebraeum* (Iweriebor et al., 2017) and *Rhipicephalus bursa* (Cicculli et al., 2019). Evidence of transstadial transmission of *E. minasensis* in *R. microplus* ticks was reported (Carvalho et al., 2016) which highlights a potential role of *R. microplus* in the transmission of this pathogen. However, *E. minasensis* DNA was detected in a bovine blood sample in Ethiopia where *R. microplus* has not been reported to occur, suggesting transmission of *E. minasensis* by other tick species different to *R. microplus* (Hailemariam et al., 2017). Notably, the prevalence of *E. minasensis* (3.2%) in ticks collected from cattle was higher than that of other well-known tick-borne pathogens including *Anaplasma ovis* (1.5%), *Babesia bigemina* (0.7%), *Babesia bovis* (0.2%), *Babesia occultans* (0.2%), and *Rickettsia massiliae* (0.2%) in Pakistan (Rehman et al., 2019). This suggests that infection by *E. minasensis* may be more frequent than generally assumed or expected. Interestingly, *E. minasensis* was found infecting cervids in areas where *A. marginale* was not detected (Lobanov et al., 2012). Coinfections between *E. minasensis* and *Anaplasma* sp. MD2010, different from *A. marginale* and all other recognized species of the genus, were also reported in cervids (Lobanov et al., 2012).

Serological diagnosis of *E. minasensis* and other pathogens of Anaplasmataceae in cattle may prove challenging because there is cross-reactivity of antibodies against the recombinant antigens (e.g. MSP5) use in the ELISA and other serological tests (Al-Adhami et al., 2011; Lobanov et al., 2012). Recently, a new TaqMan method was developed with high sensitivity and specificity to detect and differentiate *E. canis* and *E. minasensis* in infected blood (Thomson et al., 2018). Host differences, incidence of disease and geographical location of the isolates had little impact on the performance of the TaqMan method (Thomson et al., 2018). This novel assay (Thomson et al., 2018) or the use of conventional PCR for amplification and sequencing of *E. minasensis* genes 16SrRNA, *groEL*, *dsb*, *gltA* and *trp36* (Cabezas-Cruz et al., 2012) offer viable solutions to study the epidemiology and impact on animal health of this pathogen.

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<https://doi.org/10.1016/j.ttbd.2019.03.018>

Received 14 March 2019

Available online 27 March 2019

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