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Title: Molecular detection of tick-borne bacteria in ticks in southern Norway

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Objectives: Screening of a group of ticks collected from dogs in Southern Norway for the presence of tick-borne bacteria from the genera *Anaplasma, Ehrlichia, Borrelia, Bartonella, Coxiella, Rickettsia*, and *Francisella* that are responsible for bacterial arthropod-borne zoonosis.

Methods: Sample collection, preservation, and transport: Ticks were collected in the veterinary practice from dogs of varying sizes and races from a limited geographical area around Kristiansand. The ticks were preserved in 80% ethanol and sent to the Spanish Reference Center CNM (Centro Nacional de Microbiología ISCIII, Majadahonda-Spain) for processing.

DNA extraction, PCR, and genus identification: Ticks were individually crushed and lysed, and the DNA was extracted using the QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany). The DNAs were analyzed simultaneously by an in house multiplex polymerase chain reaction (PCR) and hybridization with generic and specific probes by reverse line blot (RLB) (si se ponen referencias, aquí se puede poner la de la patente 1) in the CNM, and by the Tick-Borne Bacteria Flow CHIP assay (Master Diagnostica S. L., Granada, Spain), in the reference laboratory of Master Diagnostica (Granada-Spain). This assay consists of a multiplex PCR followed by automatic hybridization onto a macroarray CHIP, based on DNA-Flow Technology (e-BRID System™). Positive and discrepant samples were confirmed by genus-specific single PCRs and sequencing in the CNM.

Results: A total of 55 tick samples were analyzed. The same results were obtained by both methods in 52 of them (38 negatives and 14 positive samples).

Three cases were positive with the in-house multiplex PCR, but negative with Tick-Borne Bacteria Flow CHIP Kit. These cases were verified by single PCR and sequencing. Two of them were confirmed positive for *Borrelia lusitaniae* and the third one for *Bartonella chomelii/ B. schoenbuchensis/ B. capreoli/ B. birtlesii*, showing very low signal intensity, the latter being not feasible for sequencing given the low amount of pathogen DNA.

Overall, 12 samples were positive for *Borrelia*, 3 for *Candidatus Neoehrlichia mikurensis*, 2 for *Rickettsia*, and 1 case was positive for *Bartonella*. Two of these samples showed co-infection, one with *Borrelia afzelii* and *Candidatus Neoehrlichia mikurensis* and the other with *Borrelia garinii* and *Anaplasma phagocytophilum.*

Conclusion: Tick borne agents in dogs and other animals have been previously described in Southern Norway. Dogs are of special importance due to ticks' preference for dogs and their proximity to humans. It is supposed that ticks affecting dogs carried the same bacteria that could affect their owners. Furthermore, emergence of co-infections with tick-borne pathogens emphasizes the need of a multiplex test to detect different pathogens simultaneously. New technologies like nucleic-based diagnostic tests for detection of tick-borne bacteria are emerging and becoming useful in the diagnosis of active infectious diseases.