PhD defence Teshale Sori Tolera

Study on Tick-Borne Pathogens and Tick-Borne Diseases Using Molecular Tools with Emphasis on Anaplasma spp. and Ehrlichia spp. in Ticks and Domestic Ruminants in Ethiopia

16 mai 201817:00
Ghent University, Faculty of Veterinary Medicine - Merelbeke

Dit is de omschrijving

Supervisors

- Prof. Dr. Pierre Dorny (Ghent University, ITM)
- Prof. Dr. Dirk Berkvens (ITM, Ghent University)
- Dr. Dirk Geysen (former ITM)
- Prof. Dr. Gobena Ameni (Aklilu Lemma Institute of Parasitology, Addis Ababa University, Ethiopia)

Summary

Although tick-borne pathogens are among the most important constraints in the domestic ruminant industry causing huge economic losses, little information is available on their occurrence and distribution in Ethiopia. Within this doctoral study, three cross-sectional epidemiological studies using molecular techniques were conducted, one on ticks collected from cattle and sheep, one on domestic ruminants and one on unfed ticks collected from the field. The survey conducted on ticks collected from cattle and sheep using a molecular analysis of 18S rDNA identified the occurrence of *Theileria buffeli/orientalis*, *Theileria velifera*, and *Theileria ovis* in *Rhipicephalus evertsi evertsi* and *Rhipicephalus decoloratus*. According to the 16s rDNA PCR and sequencing, six species of *Bartonella*, three species of *Rickettsia* (*Rickettsia africae*, *Rickettsia felis* and *Rickettsia* sp.), *Anaplasma ovis*, *Ehrlichia ruminantium*, *Ehrlichia* spp., *Anaplasma* spp. and *Borrelia burgdorferi s.l.* were identified in *Rhipicephalus* spp. This is the first report of the occurrence of *A. ovis*, *Bartonella elizabethae*, *Bartonella bovis*, *Bartonella koehlerae*, *Bartonella quintana* and *Bartonella vinsonii berkhoffii* in Ethiopia. Our finding highlights the risk of infection of animals and humans with zoonotic tick-borne bacteria in Ethiopia. At the end of the first survey, we focused on the molecular identification of *Ehrlichia* spp. and *Anaplasma* spp., in which we developed a semi-nested PCR amplifying 925bp of 16S rDNA. A pair of primers designated EBR2 and EBR3 was designed from the *Anaplasma* 16S rDNA sequences for simultaneous detection of *Ehrlichia* spp. and *Anaplasma* spp. Individual species of *Ehrlichia* and *Anaplasma* were identified by restriction with *MboII*, *HhaI* and *MspI* enzymes, including mixed infections. Analysis of *Amblyomma* spp. from various parts of Ethiopia for bacterial pathogens using this molecular method revealed the occurrence of *Anaplasma marginale*, *Anaplasma phagocytophilum, Anaplasma centrale*, *Anaplasma* (formerly *Ehrlichia*) sp. *Omatjenne* and *E. ruminantium.* By helping animal health professionals in their decision-making regarding the diagnosis and control of anaplasmosis and heartwater, this improved molecular method will help the government in establishing a disease-monitoring program in the dairy and beef industries. The second part involves surveys of *A. phagocytophilum* and *A.* sp. *Omatjenne* infection in cattle in Africa and that of *Anaplasma* spp. and *Ehrlichia* spp. in cattle, sheep and goats in Ethiopia. In the international survey, respectively 19 (2.7%) and 45 (6.5%) samples yielded positive signals for *A. phagocytophilum* and *A.* sp. *Omatjenne*. *Anaplasma* sp. *Omatjenne* was detected in all countries except Tanzania while *A. phagocytophilum* was detected only in samples originating from Ethiopia. Out of the 922 blood samples from cattle, sheep and goats from five different localities in Ethiopia analyzed by 16S rDNA PCR, 523 (56.7%) tested positive. Overall, 67.4% of cattle, 65% of sheep and 18% of goats tested positive for one or more *Anaplasma* spp. No positive result was observed for *Ehrlichia* spp. using the 16S rDNA analysis. RFLP analysis identified *A. marginale*, *A. ovis*, *A. phagocytophilum*, *A. centrale* and *A.* sp. *Omatjenne*. We conclude that infection of domestic ruminants with *Anaplasma* spp. is widespread in Ethiopia. Livestock improvement plans through introduction of improved breeds should be aware of this and take the necessary precautions to minimize losses associated with anaplasmosis. First reports of infection of domestic ruminants with *A.*

phagocytophilum, *A. ovis* and *A.* sp. *Omatjenne* are provided for Ethiopia. In addition, the occurrence of *A. phagocytophilum* together with *A.* sp. *Omatjenne* outside Europe and South Africa is made for the first time. Random samples of 493 animals were tested with a pCS20 PCR for the identification of *E. ruminantium*. Three samples from 75 (4%) cattle gave a positive result. *Ehrlichia ruminantium* was identified in five of 13 clinical cases of heartwater in dairy cows (38.46%) and 30 of 72 (41.67%) clinically affected Boer goats. Identification of ticks that carry pathogens of veterinary and public-health importance, belonging to the genus *Anaplasma*, is an initial step to identify tick vectors that play a role in the epidemiology of the diseases they cause. A molecular investigation was carried out to identify *Anaplasma* spp. carried by unfed field ticks with an emphasis on *A. phagocytophilum* and *A. ovis*. A total of 240 unfed ticks (adults and nymphs) were collected and analyzed using PCR-RFLP. *Anaplasma ovis* was identified in *R. evertsi*, *Amblyomma* spp. and *Hyalomma* spp. *Anaplasma phagocytophilum* was detected only in *Rhipicephalus pulchellus*. Our finding identified potential vectors of *A. ovis* to be further confirmed by experimental study. In conclusion, this doctoral study identified tick-borne pathogens of veterinary and public health importance in Ethiopia. An improved molecular method was developed for simultaneous detection of *Anaplasma* spp. and *Ehrlichia* spp. and it was used in epidemiological studies in domestic ruminants and ticks. A high prevalence of anaplasmosis and a low prevalence of heartwater were observed. The occurrence of infection in cattle with *A.* sp. *Omatjenne* was identified in certain African countries. Furthermore, candidate vectors of *A. ovis* were identified. Further studies on the medical significance of the identified zoonotic pathogens, search for vectors of *A. phagocytophilum* and experimental studies for the confirmation of the vectors of *A. ovis* are warranted. Lastly, an improvement of molecular methods is needed for the detection of carriers of *E. ruminantium*.

Date: 08-08-20