Abstract of Thesis/Dissertation

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Title : Molecular epidemiological studies on livestock tick-borne parasitic diseases in the Philippines (フィリピンにおける家畜のマダニ媒介性寄生虫病に関する分子疫学的研究)

Abstract

Babesiosis, theileriosis, and anaplasmosis, alongside other TBDs, are significant parasitic diseases that inflict adverse health effects on livestock animals. This impact directly translates to million worth of financial losses for livestock raisers globally. The use of molecular tools to uncover the epidemiology of TBDs has been instrumental in their prevention and control. Considering the economic impact, elucidating the presence of tick-borne infections in livestock using appropriate diagnostic tools is vital not only in improving herd health but also in formulating and implementing countermeasures, which are expected to help farmers to recuperate the losses from TBDs. In this dissertation, I was able to elucidate the major TBPs of horses (Chapter 1), goats (Chapter 2), and cattle (Chapter 3) in the Philippines through the application of molecular diagnostic tools.

In Chapter 1, the presence of equine tick-borne infections in a racehorse park from

Cavite, Philippines, was evaluated. A total of 124 thoroughbred horses specifically raised for racing were sampled. *Babesia caballi* (12.10%; 15/124), *Theileria equi* (0.81%; 1/124), *Anaplasma phagocytophilum* (10.48%;13/124), *Borrelia burgdorferi* sensu lato (Bbsl) (38.71%; 48/124), *A. marginale* (0.81%; 1/124), and *Coxiella burnetii* (0.81%; 1/124) were detected in racehorses. Of the 60 positive samples, 42 were single infections and 18 were multiple infections, the most frequent of which are coinfection with Bbsl and *B. caballi*. Sex ($p = 0.026$) was found to be a significant risk factor for *B. caballi* infection, with female horses 5.77 times more likely to be infected with *B. caballi*. Sequencing analysis revealed that seven partial 18S rRNA *B. caballi* isolates shared 98.63–100% identity and were classified as genotype A, whereas the single *T. equi* sequence had 99.77% identity with GenBank isolates and was confirmed as genotype E. Eight *Anaplasma* 16S rRNA partial sequences were highly identical to *A. phagocytophilum* and *A. ovis*, while partial sequences of *Borrelia* 5–23S rRNA were most closely related to *Bo. japonica* and *Bo. garinii*-like isolates. The first molecular detection of *Borrelia* and *Anaplasma* is reported in this study, as well as the first genotyping of *B. caballi* and *T. equi* in racehorses in the Philippines.

In Chapter 2, a total of 396 goat samples were collected from six Philippine provinces and were molecularly screened for the presence of *Babesia/Theileria* and *Anaplasma*. A detection rate of 77.02% (305/396) and 38.64% (153/396) were noted for the respective TBPs. Six samples were positive for *B. ovis* (1.52%). Sex and age group were associated with higher *Babesia/Theileria* and *Anaplasma* detection rates, respectively, while significantly higher rates for both pathogens were observed in exotic goat breeds. The representative *Babesia/Theileria* sequences shared 89.97–97.74% identity and were most closely related to *T. orientalis*, *T. annulata*, and *Theileria* sp. On the other hand, *Anaplasma* 16S rRNA sequences were related to *A. odocoilei*, *A. platys*, and *A. phagocytophilum*. This is the first molecular identification of *B. ovis*, *Theileria* spp., and *Anaplasma* spp. in goats from the Philippines.

In Chapter 3, a new molecular tool was evaluated for the characterization of piroplasma species diversity in bovines. Of 162, 58.64% tested positive for piroplasma using a conventional

RLB-PCR assay that targets the V4 hypervariable region of the 18S rRNA gene. The positive cattle samples were from Bohol (83.33%; 20/24), Cavite (70.97%; 44/62), and Cebu (40.79%; 31/76). By leveraging the AMPtk pipeline, the merged reads generated a total of 2,179 ASVs. The BLAST non-redundant database assigned the taxonomy of 175 ASVs, which were then dereplicated into 97 ASVs. Further filtering yielded a final count of 79 distinct ASVs. The taxonomy hits of the 79 ASVs corresponding to 10 species were *B. bovis* (n = 37), *B. bigemina* (n = 18), *T. orientalis* (n = 13), *Babesia* sp. (n = 3), *Hepatozoon canis* (n = 2), *Sarcocystis cruzi* (n = 2), *T. annulata* (n = 1), *T. equi* (n = 1), *T. mutans* (n = 1), and *Theileria* sp. Thung Song (n = 1). Based on the phylogenetic analysis, ASVs assigned to *B. bovis* and *Babesia* sp. showed three major subclades and were 90.76–100% identical with *B. bovis* isolates deposited in GenBank. On the other hand, the evolutionary inference suggested that most of the *B. bigemina* and *Babesia* sp. ASVs were phylogenetically distinct from previously reported isolates, as evidenced by the strong statistical support, and shared 90.23–99.77% identity with isolates from the database. Thirteen *T. orientalis* ASVs (91.72–100% identical to other *T. orientalis* isolates) clustered in the *T. orientalis* complex subclade, 4 of which formed a subgroup with high nodal support. As anticipated, ASVs taxonomically recognized as *T. annulata*, *T. equi*, and *T. mutans* were separated into groups based on the species to which they belong. Furthermore, the *Hepatozoon* ASVs were distinctly divergent from previously detected Philippine canine *H. canis* isolates, while phylogenetic analysis revealed that *Sarcocystis* ASVs were closely related to bovine *S. cruzi* isolates from Malaysia and India.

The fundamental purpose of this research was to map out the different TBPs infecting economically important livestock in the Philippines. The presented findings from the three chapters of this dissertation attest to the attainment of this purpose. The circulating TBP populations and the extent of tick-borne infections in horses, goats, and cattle in the Philippines were revealed through the use of molecular detection tools, proving the applicability of these platforms in identifying tick-borne infections in different livestock. These results are vital in ascertaining the TBD status in the Philippines, where infections are widely present but often

overlooked and neglected. The findings from these studies shall be beneficial in crafting and

implementing livestock tick and TBD control and prevention programs in the country.