Studies on the development of diagnostic methods and chemotherapeutics of babesiosis

その他（別言語等）のタイトル	バベシア病の診断法と化学療法の開発に関する研究
著者（英）	Guswanto
学位名	博士（畜産衛生学）
学位授与機関	帯広畜産大学
学位授与年度	2017
学位授与番号	10105甲第81号
URL	http://id.nii.ac.jp/1588/00001397/

Form No.5

Abstract of Thesis/Dissertation

Applicant : Guswanto

Master's/Doctoral Program in Animal and Food Hygiene

Graduate School of Animal Husbandry

Obihiro University of Agriculture and Veterinary Medicine

Student ID: 26602

Signature of Applicant:

Title : Studies on the development of diagnostic methods and chemotherapeutics of babesiosis

(バベシア病の診断法と化学療法の開発に関する研究)

Abstract

Babesia parasites have been found for more than a century and cause a significant burden in the livestock industry and emerging disease in humans. The distribution of the disease remains as wide as the occurrence of their tick vectors. This study aimed to evaluate a reliable diagnostic method, such as immunochromatographic test (ICT) strips, for serological detection of bovine babesiosis in field samples. In an extended sampling coverage in Indonesia, ICT strips, an enzyme-linked immunosorbent assay（ELISA）, and nested PCR were employed for the detection of *B. bovis* and *B. bigemina* in cattle. Another objective of this study was to evaluate the inhibitory effect of 17-dmag alone and in combination with diminazene aceturate and atovaquone on the growth of *Babesia* and *Theileria* parasites *in vitro* and *in vivo*.

In chapter 1, three types of ICT strips were prepared for the detection of an antibody response against the spherical body protein 4 (SBP-4) of *Babesia bovis* (*bov*ICT), the C-terminal-truncated rhoptry-associated protein 1 (rRAP1/CT17) of *B. bigemina* (*big*ICT), and a combination of both proteins (dual-ICT). The evaluation of their performance was conducted using a confirmed positive and negative serum panel for *B. bovis* and *B. bigemina*. Together with ELISA, the ICT strips were applied to determine the seroprevalence of bovine babesiosis in Western Java, Indonesia. Among 991 serum samples, 28.4%, 25.3%, and 24.5% of cattle were detected to be seropositive for *B.*

bovis infection using ELISA, *bov*ICT, and dual-ICT, respectively. *B. bigemina* seropositivity was detected in 27.1%, 24.2%, and 22.8% of samples using ELISA, *big*ICT, and dual-ICT, respectively. The comparison of ICT and ELISA results using field serum samples showed good agreement with Kappa values were more than 0.7. The application of ICT strips is preferable in field situations where rapid diagnosis is required. Furthermore, the data showed the current seroprevalence of bovine babesiosis in Western Java, Indonesia, and efficient control strategies are needed to reduce economic losses due to the disease.

In chapter 2, an extended sample collection was conducted in several islands of Indonesia. Subsequently, molecular and serological detections of bovine babesiosis were performed on 487 blood samples. The presence of antibodies and current infections of *B. bovis* and *B. bigemina* were determined using ELISA, ICT, and nested PCR (nPCR) targeting *B. bovis* sbp4 and *B. bigemina* rap-1a genes. Among 487 samples, ELISA, single-ICT, dual-ICT, and nPCR detected positive *B. bovis* in 340 (69.8%), 317 (65.1%), 307 (63.0%), and 247 (50.7%) of the samples, respectively. For *B. bigemina*, 134 (27.5%), 130 (26.7%), 127 (26.1%), and 93 (19.1%) samples were positive, respectively. Furthermore, mixed infections were found in 125 (25.7%), 113 (23.2%), 109 (22.4%), and 52 (10.7%) samples, respectively. The obtained nucleotide sequences of *B. bovis* sbp4 and *B. bigemina* rap-1a genes in this study showed a high homology with other isolates from many countries, and the identities among Indonesian isolates were 94 to 100%. These data revealed the current distribution of *B. bovis* and *B. bigemina* infection in cattle in Indonesia, and we found that the positive rate of *B. bovis* is high in most sampling locations while B. bigemina is lower. The data presented in this study are necessary to develop an effective strategy for controlling the disease in that country.

In chapter 3, an Hsp90 inhibitor, 17-dmag, was evaluated for its inhibitory effect on five *in vitro* cultures of *Babesia* and *Theileria* species, *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi*, and *T. equi*, followed by the growth inhibition assay of a *B. microti*–infected mouse model. 17-dmag showed the inhibition in all of the species tested. The half maximum of inhibition concentration (IC_{50}) of 17-dmag on the *in vitro* growth of *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi*, and *T. equi* was 77.6 ± 2.9, 62.4 ± 1.9, 173.0 ± 10.9, 88.5 ± 9.6, and 307.7 ± 7.2 nM, respectively. The toxicity assay on MDBK and NIH/3T3 cell lines showed that 17-dmag affected the growth of cells with half maximum effective concentrations (EC_{50}) of 15.5 ± 4 μM and 8.8 ± 2 μM, respectively. Since the IC_{50} was much lower on the parasites, the selectivity index was high in all tested species

compared to the host. Furthermore, the two-drug combination of 17-dmag with diminazene aceturate (DA) and atovaquone (AV) showed synergism or addition *in vitro*. In the mouse model, 17-dmag at the concentration of 30 mg/kg BW effectively inhibited the growth of *B. microti*. Moreover, the combination of a half dose of 17-dmag and DA showed a comparable inhibition if used at the full dose. Taken together, this indicates that 17-dmag is a potent drug in treating babesiosis. Its toxicity can be reduced by combining it with other effective drugs, such as DA and AV. The data warrant further evaluation of 17-dmag as an anti*Babesia*l and as a treatment option in combination with atovaquone for the treatment of human babesiosis.

To sum up, these studies are reporting the current development of diagnostic methods for bovine babesiosis. The ICTs in this study, even though their sensitivity is slightly lower than that of ELISA, are useful for the detection of *B. bovis* and *B. bigemina* antibodies, e specially in field applications. These data also reveal the current distribution of bovine babe siosis in cattle in Western Java, and the extent sampling areas in Indonesia. The results in dicate that bovine babesiosis might be a significant economic burden on the Indonesian liv estock industry. The development of effective control strategies for bovine babesiosis in tha t country could be benefited by the data provided in this study. Furthermore, 17-dmag is a potent anti*Babesia*l drug. It specifically inhibits the growth of *Babesia* and *Theileria* parasit es both *in vitro* and *in vivo*. Its toxicity could be reduced by combination therapy. The dev elopment of diagnostic methods and chemotherapeutics for bovine babesiosis is still in prog ress, and further improvements are necessary to combat this important disease.