Abstract: Enoxacin is a broad-spectrum 6-fluoronomphthyridine antibacterial agent (fluoroquinolones) structurally related to nalidixic acid used mainly in the treatment of urinary tract infections and gonorrhea. Also it has been shown recently that it may have cancer inhibiting effect. The primary antibabesial effect of Enoxacin is due to inhibition of DNA gyrase subunit A, and DNA topoisomerase. In the present study, enoxacin was tested as a potent inhibitor against the in vitro growth of bovine and equine Piroplasms. The in vitro growth of five Babesia species that were tested was significantly inhibited (P< 0.05) by micro molar concentrations of enoxacin (IC$_{50}$ values = 13.5, 7.2, 7.5 and 24.2 μM for Babesia bovis, Babesia bigemina, Babesia caballi, and Theileria equi, respectively). Enoxacin IC$_{50}$ values for Babesia and Theileria parasites were satisfactory as the drug is potent antibacterial drug with minimum side effects. Therefore, enoxacin might be used for treatment of Babesiosis and Theileriosis especially in case of mixed infections with bacterial diseases or incase of animal sensitivity against diminazin toxicity.

Materials and methods:
Parasites: The Texas strain of B. bovis, the Argentine strain of B. bigemina the USDA strain of B. caballi, Theileria equi, and the Munich strain of B. microti were used in this study.
Culture conditions: The Babesia parasites used in this study were maintained in bovine or equine red blood cells (RBCs) using a microaerophilic stationary-phase culture system. Briefly, Medium 199 was used for B. bovis, B. bigemina, and T. equi, while RPMI-1640 was used for B. caballi (both from Sigma-Aldrich, Tokyo, Japan).
Chemicals reagents: Enoxacin was purchased from Sigma–Aldrich, Tokyo, Japan and used as a test drug. A working stock solution of 100 μM Enoxacin dissolved in (DMSO) in vitro growth inhibition assay: The inhibitory effect of enoxacin on the growth of Babesia parasites was tested using an assay previously described with slight modification. Parasite-infected RBCs will be diluted with uninfected RBCs to obtain an RBC stock supply with 1% parasitemia. Twenty μl of RBCs with 1% parasitemia will be dispensed into a 96-well microtitre plate (Nunc, Roskilde, Denmark) with 200 μl of the culture medium containing the indicated concentration of Enoxacin (0.1, 1, 10, 15, 25 and 50 μM) for B. bovis and (0.1, 1, 0.5, 10, 15, 25 and 50 μM) for other Babesia species, and then incubated at 37 °C in a humidified multi-gas water-jacketed incubator.
Viability test: After the 4th day of treatment, 6 μl of each of the control and drug-treated (at the various indicated concentrations) RBCs will be mixed with 14 μl of parasite free RBCs and suspended in fresh growth medium without Enoxacin supplementation. The plates will be incubated at 37 °C for the next 10 days.