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| Title | INFORMATION: Theses for the degree of Doctor of Philosophy |
| Citation | Japanese Journal of Veterinary Research, 62(4), 193-203 |
| Issue Date | 2014-11 |
| Doc URL | http://hdl.handle.net/2115/57509 |
| Type | bulletin (other) |
| File Information | JJVR_62.4_Information.pdf |



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Hokkaido University conferred the degree of Doctor of Philosophy on September 25, 2014 to 8 recipients.

The titles of thesis and other information are as follows:

Analysis of mast cells in the neonatal ovary of MRL/MpJ mice —unique immune cells participating in early follicular development—

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Mast cells (MCs) reside in most tissues and act as sentinel cells in both innate and adaptive immunity. In several mammalian species including human, MCs are also present in the adult ovaries, and previous studies indicated that MCs play some roles in reproductive functions. Although a few MCs are found in the neonatal ovaries of ICR and C57BL/6N mice, the functional relationship between MCs and the perinatal ovary is unclear. In this study, the author found numerous ovarian MCs (OMCs) in neonatal MRL/MpJ mice, analyzed them with follicular development, and clarified the factors affecting their appearance.

CHAPTER I: Among 11 different mouse strains, MRL/MpJ mice possessed the greatest number of OMCs. In MRL/MpJ mice, the OMCs were most abundant at postnatal day 0, and tended to localize adjacent to the surface epithelium. Immune cells such as macrophages, B cells, T cells, neutrophils and eosinophils were rarely observed in the ovary of MRL/MpJ and C57BL/6N mice at postnatal day 0. In MRL/MpJ mice, the number of OMCs within the oocyte nests was significantly higher than in the other strains, and some OMCs directly contacted the compressed and degenerated oocytes. In contrast, the OMCs localized outside of the oocyte nests rarely contacted with the primordial and primary follicles. In MRL/MpJ mice, the number of

oocytes in the nest was significantly lower than in the other strains, and the number of oocytes showed a positive correlation with the number of OMCs. The gene expression of a MC marker also correlated with the expression of an oocyte nest marker. Furthermore, the expression of follicle developmental markers was significantly higher in MRL/MpJ mice than in C57BL/6N mice. These results indicate that the appearance of OMCs is a unique phenotype of neonatal MRL/MpJ mice, and OMCs would participate in the nest breakdown and contribute to the acceleration of the early follicular development in MRL/MpJ mice.

CHAPTER II: The author investigated the factors regulating the appearance of neonatal OMCs using the delayed parturition model and the progeny of the crosses between MRL/MpJ and C57BL/6N strains. In MRL/MpJ fetuses for which parturition was delayed until embryonic day 21.5, the number of OMCs was significantly higher than in age-matched controls at postnatal day 2. F1 neonates had less than half the number of OMCs than MRL/MpJ mice. Interestingly, MRLB6F1 had more neonatal OMCs than B6MRLF1, although they were distributed over comparable areas. Quantitative trait locus analysis using N2 backcross progeny revealed two significant loci on chromosome 8: *D8Mit343-D8Mit312* for the number of OMCs and *D8Mit86-*

D8Mit89 for their distribution, designated as *mast cell in the ovary of MRL/MpJ 1 (mcom1)* and *mcom2*, respectively. Among MC migration-associated genes at *mcom1* locus, ovarian expression of chemokine (C-C motif) ligand 17 was significantly higher in MRL/MpJ than in C57BL/6N mice, and positively correlated with the expression of OMC marker genes. These results indicate that the appearance of neonatal OMCs in MRL/MpJ mice is controlled by environmental factors and filial genetic factors, and that the abundance and distribution of OMCs are regulated by independent filial genetic elements.

In mice just after birth, the oocyte nests

break apart into individual cells and become primordial follicles. During this process, called nest breakdown, only a subset of oocytes survives and the remaining oocytes die. The author's results suggest that the numerous OMCs in MRL/MpJ mice participate in the nest breakdown, and contribute to progressed early follicular development, under the control of both the environmental factors and the genetic factors. Therefore, the author concluded that MC is a unique cell relating to the progression of early follicular development, and MRL/MpJ mice would be useful to clarify the close relationship between immune system and reproductive system through MCs.

The original papers of this thesis appeared in *PLoS One* **8**: e77246 (2013) and **9**: e100617 (2014).

Development of the new protocol for habitat modeling of urban red fox to improve *Echinococcus multilocularis* control strategy

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Echinococcus multilocularis Leuckart, 1863 is a parasite which will cause the human alveolar echinococcosis (HAE), one of the most serious helminthic zoonoses. HAE is spreading widely in the northern hemisphere and the number of cases has been increasing in recent years. The main transmitter of the parasite to human is the red fox, *Vulpes vulpes* Linnaeus, 1758. Deworming wild red foxes by baiting with the anthelmintic praziquantel is being established as a preventive technique to control the parasite. Improvement of the cost-benefit performance of baiting treatment is required urgently to maintain the efficacy of deworming.

In the present study, the efficient sites to be

delivered the anthelmintic baits in urban area were determined by habitat modeling of red fox den sites. Habitat modeling of the generalist (the species which does not have any critical requirements for environmental resources) such as red foxes is considered to be difficult by existent methods, so the new protocol suitable for urban red foxes was developed.

The study was conducted in urban regions of Obihiro (about 59.8 km²) and Sapporo cities (about 367.9 km²) in Hokkaido, Japan, in which red fox populations have been established. The two cities have different degrees of urbanization. Sampling of fox dens location was conducted by thorough field exploring on the basis of the

information from citizens. A total of 35 fox dens in Obihiro study area (from 2002 to 2004) and 65 dens in Sapporo (from 2004 to 2007) were found. As against the points with dens “present”, control points were dotted randomly on the analytical base map as “absence” data (120 points in Obihiro; 730 points in Sapporo). The base map was customized for analyzing red fox ecology inhabiting urban areas by modifying existent numerical information maps, residential maps, and aerial photographs, which consists of nine micro-habitat categories: “wide road”, “narrow road”, “occupied building”, “vacant building”, “water place”, “riverbed”, “farmland”, “green covered area”, and “blank space”. These nine categories were set based on the previous reports on red fox habitat and to express the urban landscape. Den site selection modeling was conducted by use of the materials above for the red fox population in each of Obihiro and Sapporo study areas. The modeling protocol was designed to detect the best combination of the key environmental factors and the key spatial scale that foxes pay attention to (named “heeding range”) when they select den sites, although the existent method can only detect the key environmental factors. All possible models were generated using logistic regression analysis, with “presence” or “absence” of fox den as the objective variable, and the percentages of the nine micro-habitat categories as predictor variables to detect the key environmental factors. This procedure was conducted for each of ten sizes of concentric circles (100–1000 m) from dens and control points to detect the most affecting circle size, that is, the key spatial scale. Out of all models generated, the best model was selected using Akaike’s information criterion (AIC) inspection. This procedure was done in the each of the two study areas.

Established models suggest that requirements for denning are low percentages of wide roads, narrow roads, and occupied buildings, but high

percentages of green covered areas within the circle of 500 m radius in Obihiro fox population; low percentages of wide roads, occupied buildings, but high percentages of riverbeds and green covered areas within 300 m radius in Sapporo. The difference in size of the key spatial scale between the two cities populations may come from the differences in their sensitivities to the surrounding environments. Both populations focused on the densities of wide roads, occupied buildings and green covered areas in common for their den sites. Accuracy of these models were inspected by area under the curve (AUC) of receiver operating characteristic (ROC) curve, and the values showed that those models have sufficiently high accuracy (AUC = 0.987 for Obihiro model; 0.995 for Sapporo model). Besides, prediction performances were evaluated by calculating the rates of concordance between observed and predicted values, and the rates were also sufficiently high (92.3% for Obihiro model; 99.2% for Sapporo model).

In conclusion, the den site selection models of urban red foxes have successfully been established, and the new modeling protocol has also been developed. The established model could determine the efficient sites for delivering baits, and it will improve the cost-benefit performance of the deworming by anthelmintic baiting campaign. Although it is generally considered that habitat selection modeling for generalist species is difficult, but the protocol developed in this study enabled it by use of the suitable variables for the target species. This modeling approach can be adopted for every type of habitat, including urban, suburban, rural, or primitive landscapes. The variation of the models shown in this study suggests the necessity of accumulating models for various types of cities in order to reveal the patterns of the model. It will enable us to perform rapid and efficient deworming campaign.

Application of contrast-enhanced ultrasonography in diagnosis of canine pancreatic disease

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Acute pancreatitis (AP) is a common disease of the canine exocrine pancreas and accurate noninvasive diagnosis is challenging. Contrast-enhanced ultrasonography (CEUS) is a major breakthrough for ultrasound imaging and can assess organ perfusion. This study was performed in three stages to determine the feasibility of using quantitative CEUS to diagnose canine AP.

Chapter 1 aimed to (1) characterize contrast-enhancement of the pancreas using bolus injection and continuous infusion of contrast agent and (2) to assess if continuous infusion can prolong pancreatic enhancement. CEUS of the pancreas were performed in eight dogs, and time-intensity curves were generated. Four perfusional parameters were measured for statistical analysis: time to initial up-slope (TTU), peak time (Tp), time to wash-out (TTW), and peak intensity (PI). Median pancreatic contrast-enhancement was prolonged by continuous infusion from 11 (range, 10 to 23) s to 205 (170 to 264; $P < 0.01$) s. Median PI was higher during bolus injection when compared to continuous infusion (100.9 [80.2 to 124.3] MPV versus 77.6 [58.2 to 99.5] MPV; $P < 0.05$). Prolonged continuous imaging was afforded by continuous infusion. PI was slightly lower in continuous infusion, but offered adequate imaging subjectively.

Chapter 2 aimed to investigate the feasibility of CEUS in detecting pancreatic perfusional changes in cerulein-induced canine AP. Six dogs received 2 hours of IV infusion with 7.5 $\mu\text{g}/\text{kg}/\text{h}$ of cerulein diluted in saline. As control, all dogs received 2 hours of IV infusion of saline two weeks before cerulein infusion. CEUS of the pancreas were performed before (0 hour), and at

2, 4, 6 and, 12 hours after saline and cerulein infusion. Perfusion parameters TTU, Tp, TTW, PI, and area under the curve (AUC) were compared between saline and cerulein infusion. In cerulein-induced AP, PI increased at 2 and 4 hours when compared to 0 hour, and at 2, 4, and, 6 hours when compared to control. AUC increased at 4 hours when compared to 0 hour, and at 2 and 4 hours when compared to control. TTW was prolonged at 4 hours when compared to control. PI, AUC, and TTW can provide useful information in differentiating AP from normal pancreas. Cerulein-induced AP was characterized by prolonged hyperechoic enhancement on CEUS.

Chapter 3 aimed to investigate the feasibility of CEUS to detect pancreatic perfusion changes in naturally occurring pancreatitis. Twenty-three dogs diagnosed with pancreatitis were prospectively enrolled. Pancreatic CEUS were performed and perfusion parameters (TTU, Tp, TTW, PI, AUC) were compared to normal controls. Tp of the pancreatitis group was prolonged when compared to controls ($P < 0.001$). PI and AUC were increased when compared to controls ($P < 0.01$ and $P < 0.05$, respectively). CEUS can detect pancreatic perfusion changes in naturally occurring pancreatitis and was characterized by delayed peak with prolonged hyperechoic enhancement on CEUS.

In conclusion, pancreatic CEUS protocol was established in this study, and detection of pancreatic perfusion changes both in experimentally induced and naturally occurring pancreatitis were demonstrated. CEUS is potentially useful as a new noninvasive diagnostic tool in diagnosing naturally occurring canine pancreatitis.

The original paper of this thesis appeared in *J. Vet. Med. Sci.*, **75**: 1601–1607 (2013), *J. Vet. Intern. Med.*, **28**: 496–503 (2014) and *J. Vet. Intern. Med.*, (doi: 10.1111/jvim.12470.) Epub ahead of print (2014).

Studies on the antigenic characteristics of tick-borne flaviviruses and their application

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TBEV and OHFV are both members of the TBE serocomplex although TBEV causes encephalitis while OHFV causes hemorrhagic fever syndrome. TBEV causes over 10,000 cases of encephalitis annually, with an endemic area extending from Western Europe to East Asia. The high public health significance of the virus necessitated the development of TBE vaccines. The development of a vaccine against OHF is economically unfeasible due to the lower prevalence of the disease. However, effective protection against OHF is required during outbreaks. The feasibility of a cross-protective flavivirus vaccine provides a cost effective method for OHF prevention.

The E protein of flavivirus is highly antigenic and is the major target for antibodies. The cross-neutralization of flaviviruses within a serocomplex is dependent on the degree of E protein similarity. Despite the obvious differences in disease manifestation, the two viruses share over 80% E protein homology. The high amino acid similarity suggested that a vaccine against TBE could cross-protect against OHF. In the chapter 1, the potential of commercially available TBE vaccines to protect against OHFV infection was investigated. Neutralization tests performed on sera from OHFV and TBEV-infected mice showed that neutralizing antibodies were cross-reactive. The GMT of antibodies against TBEV and OHFV from TBEV-infected mice were similar. However,

the levels of anti-TBEV antibodies in OHFV-infected mice were significantly lower than the levels of anti-OHFV antibodies in the same animals. In mouse vaccination and challenge tests, the TBE vaccine provided 100% protection against OHFV infection. In addition, eighty-six percent of vaccinees seroconverted against OHFV following complete vaccination, and the GMT of neutralizing antibodies against OHFV were comparable to those against TBEV. These data suggest that the tick-borne encephalitis vaccine can prevent Omsk hemorrhagic fever virus infection.

TBEV is one of the most important vector-borne viral pathogens and the incidence of TBE has been increasing. Integrated surveillance of the vector and the virus in reservoir hosts and humans can identify TBE endemic risk areas and provide information for efficient prevention and control measures. Surveillance of the virus in reservoir species and humans requires cheap, reliable, and quick diagnostic tests. However, the production of commercial TBE ELISA kits is expensive as it requires the high biosafety facilities for the production of the inactivated whole virus antigen. The E protein is highly immunogenic and is therefore an important antigen for flavivirus diagnosis and vaccine development. In chapter 2, the applicability of E-Fc fusion proteins as antigens for TBE serodiagnosis was investigated. The E protein

ectodomain was fused to the Fc domain of rabbit IgG and the recombinant protein was expressed in mammalian cells. The recombinant E-Fc protein retained reactivity with both anti-TBEV and rabbit anti-IgG antibodies. The lack of cross-reactivity of the E-Fc antigen with mouse and human anti IgG antibodies suggested that the antigen could be useful in detecting anti-TBEV

antibodies in multiple species. The E-Fc proteins were then used to develop an ELISA to detect TBEV antibodies. The E-Fc ELISA had high sensitivity and specificity in detecting TBEV antibodies in rodent and human sera. The results suggest this recombinant protein would be a good alternative to inactivated whole virus antigens in TBEV surveillance.

The original papers of this thesis appeared in *Microbiol. Immunol.*, **58**: 112 (2014) and *Diagn. Microbiol. Infect. Dis.*, **78**: 373 (2014).

Levels and effects of organochlorine pesticides and heavy metals in aquatic ecosystem from the Rift Valley region, Ethiopia

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Organochlorine pesticides (OCPs) and heavy metals are ubiquitous and persistent contaminants with high bioaccumulation ability, and as a consequence high concentrations can be found in environmental and biota samples. In particular, species situated high on the food chain can accumulate very high concentrations of these environmental pollutants. OCPs and heavy metals are originated from natural (for heavy metals), and anthropogenic (for OCPs and heavy metals) sources. They have been associated with various toxic effects in humans and wildlife such as endocrine disruption, cancer, poisoning, serious illness and even death. Although the use of OCPs has been banned or restricted in developed nations, they are still being used for agricultural and public health purposes in developing countries like Ethiopia. Especially, one of the most controversial pesticide, DDT is being widely used in Ethiopia for agricultural and vector control. Furthermore, Ethiopia is

burden with accumulated stockpiles of pesticides the so called “obsolete pesticides” since the first imported in the 1960s. The Ethiopian Rift Valley region which encompasses seven lakes is an important area for agricultural, commercial and industrial development of Ethiopia. At the same time it is one of the most environmentally vulnerable areas in Ethiopia. OCPs and heavy metals pollution in Ethiopia is anticipated mainly from anthropogenic sources.

In response to these concerns, the present study aimed to elucidate the bioaccumulation profiles and ecological risk assessment of OCPs and heavy metals from two Ethiopian Rift Valley Lakes—Lake Awassa and Lake Ziway. Twenty five surface sediment samples and three fish species; Tilapia (*Oreochromis niloticus*), Catfish (*Clarias gariepinus*) and Barbus (*Barbus intermedius*) from Lake Awassa, and five fish species; Tilapia, Zillii (*Tilapia zillii*), Carp (*Carassius* spp.), Catfish and Barbus and four bird

species; Hamerkop (*Scopus umbretta*), African sacred ibis (*Threskiornis aethiopicus*), Marabou stork (*Leptoptilos crumeniferus*) and Pelican (*Pelecanus onocrotalus*) from/around Lake Ziway were collected for this research study.

DDTs, HCHs, heptachlors, and chlordane compounds were the most predominant and ubiquitous residues. In general, the contamination levels of OCPs on both lakes were dominated by DDTs, attributing to their current use in vector control, illegal usage for agriculture, contamination from past usage and spills from obsolete pesticides. In sediment samples, the levels of DDT metabolites (DDE and DDD) were exceeded the sediment quality guideline values, and thus identified as chemicals of potential ecological concern in Lake Awassa. There were significant differences in OCPs levels among the studied fish species in the present study. The carcinogenic hazard ratio exceeded the threshold value of one for most of the studied fish species. Cumulative daily consumption of fish showed a potential concern on human health problem a lifetime cancer risk of greater than one in a million. High burden of DDTs was observed in all bird species under study. The level of DDE

could pose deleterious effects on survival and/or reproduction in all bird species. According to the levels of heavy metals, they were found at low concentrations except to mercury (Hg), which exceeded the permissible limit (0.3 Hg $\mu\text{g/g}$ ww) in *B. intermedius* fish species from Lake Awassa.

In general, my findings call for urgent action to reduce the level of OCPs' exposure and their effects on wildlife and human health. The low level of awareness in the study area and the public health and environmental consequence resulting from the misuse of pesticides is alarming. Therefore, there should be an integrated effort from governmental and non-governmental organizations in order to plan, tackle and control the use of pesticides effectively under the requirements of the Stockholm Convention, especially in relation to the misuse of DDT in agriculture. Although DDT is a low cost antimalarial tool, the possible adverse ecotoxicological effects through IRS must be carefully weighed against the benefits to malaria control. Routine ecotoxicological risk assessment of persistent organic pollutants in the Ethiopian Rift Valley region seems necessary.

The original papers of this thesis appeared in *Chemosphere*, **91**: 857–863 (2013), *Environ. Sci. Pollut. Res. Int.*, **20**: 8663–8671 (2013), *Ecotoxicol. Environ. Saf.*, **106**: 95–101 (2014) and *Environmental Pollution*, **192**: 121–128 (2014).

The role of sika deer (*Cervus nippon yesoensis*) in the transmission of *Borrelia* spp. in Hokkaido, Japan

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The intent of this research was to describe the role of deer in the transmission of different species of *Borrelia* spp. Sika deer (*Cervus nippon yesoensis*) are the hosts of ticks of various species

and stages in Hokkaido. Deer overpopulation has caused problems with forestry and agricultural production. The potential of deer involvement in zoonoses is also being watched. In this study,

deer caught in the nuisance control scheme and ticks collected from deer and the field were tested to detect *Borrelia* spp.. These results were analyzed for the following two subjects.

Firstly, a survey was held related to a relapsing fever *Borrelia* sp. recently found in Hokkaido. A relapsing fever *Borrelia* sp. similar to *Borrelia lonestari* was detected from wild sika deer and *Haemaphysalis* ticks in the eastern part of Hokkaido, Japan. The total prevalence of this *Borrelia* sp. in tested deer blood samples was 10.6% using conventional PCR and real-time PCR methods. The prevalence was significantly higher in deer fawns compared to adults (21.9% and 9.4%, respectively). Additionally, there was a significant regional difference between two sampling areas, Shiretoko and Shibetsu with 17% and 2.8% prevalence, respectively. Regional differences were also found in tick species collected from the field and on deer. In the Shiretoko region, *Haemaphysalis* spp. were more abundant than *Ixodes* spp., while in Shibetsu, it was the opposite. Using real-time PCR analysis, *B. lonestari*-like was detected from 2 out of 290 adult *Haemaphysalis* spp. ticks and 4 out of 76 pools of nymphs. This is the first report of a *B. lonestari*-like organism in *Haemaphysalis* spp.

ticks, and the first phylogenetic analysis of this *B. lonestari*-like organism in Asia. Based on this result, *Haemaphysalis* spp. are the most likely candidates to act as a vector for *B. lonestari*-like; furthermore, regional variation of *B. lonestari*-like prevalence in sika deer may be dependent on the distribution of these ticks.

The second survey was on Lyme disease borreliae in deer with an aspect different from the common vector-reservoir relationship. To determine whether and which types of borrelial spirochetes are extracted from *Ixodes persulcatus* ticks during feeding on sika deer, the infection rates of *Borrelia* spp. among *I. persulcatus* were compared between the two groups; the feeding ticks on deer and the questing flat ticks in the field. Lyme disease *Borrelia* spp. was detected in about 42% of adult and 6% of nymph questing ticks, while it was 3% and 1% from each group of feeding ticks, respectively. In contrast, the infection rate of *B. miyamotoi*, which belong to RF borreliae (sharing same vector), was not significantly different between groups, just below 1% among both feeding and questing ticks. Therefore, it could be said that sika deer may be the zooprophylactic host for ticks harboring Lyme disease borreliae in Hokkaido.

The original papers of this thesis appeared in *Ticks Tick Borne Dis.*, 5: 841-847 (2014).

Molecular epidemiological study of protozoan and other zoonotic diseases from two countries in Africa

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Human African trypanosomiasis (HAT), also known as sleeping sickness, is a neglected tropical disease that impacts 70 million people distributed over 1.55 million km² in sub-Saharan

Africa. *Trypanosoma brucei gambiense* accounts for almost 90% of the infections in central and western Africa, the remaining infections being from *T. b. rhodesiense* in eastern Africa. Furthermore,

the animal diseases caused by trypanosomes inflict major economic losses to countries already strained. The parasites are transmitted to the mammalian hosts through the bite of an infected tsetse fly. Additionally, zoonoses are infections or diseases that can be transmitted directly or indirectly between animals and humans. This study assessed the molecular epidemiology of human and animal trypanosomes, in addition to zoonotic pathogens in non-human primates in Zambia.

The first chapter of this thesis describes results of molecular epidemiological study on trypanosomiasis which were carried out in two tsetse-infested areas of Ghana. The samples included tsetse flies, and cattle and pig blood, and were analyzed by using multiple polymerase chain reaction tests. *Trypanosoma vivax* was the most prevalent trypanosome species, followed by *T. congolense* and *T. brucei brucei*. Two subspecies causing HAT, *T. b. gambiense*, and *T. b. rhodesiense* were not detected in animals and flies in this study, which confirms that the country having been formally a HAT focus has been free of HAT since 2000. The results in this study may be reflected by the fact that *T. vivax* can be mechanically transmitted by biting flies in addition to biological transmission by tsetse fly, hence its distribution outside the tsetse fly belt of Africa.

The second chapter describes results on the genetic characterization of *T. vivax* strains from

different geographical regions based on sequence comparison of Cathepsin L-like gene. *T. vivax* from Ghana clustered with West African and South American strains while *T. vivax* from Zambia clustered with East and Southern African strains. These results revealed genetic diversity of *T. vivax* in Africa.

In the third chapter, molecular epidemiological studies on zoonotic pathogens in non-human primates in Mfuwe in South Luangwa National Park, Zambia were carried out. This area is a HAT endemic focus with wildlife-livestock-human interface, hence the risk for zoonotic disease transmission is very high. Three species of zoonotic pathogens, *Rickettsia africae*, *Anaplasma phagocytophilum* and *Babesia microti* were detected among 9 pathogenic species/genera tested by PCR. These zoonoses detected in Zambia could be endemic in Zambian primates and possibly transmitted to humans but simply misdiagnosed as malaria due to their febrile nature.

Zoonoses in Africa are not just an African problem, since recent studies reveal an increase in these zoonotic infections in non endemic countries, especially in returning tourists from African national parks. Therefore, zoonotic disease control requires a multi-sectoral approach involving participants from the health, veterinary, entomology and environment professions because zoonosis transmission involves interaction between the pathogen, host, vector and environment.

Epidemiology, biological effects and treatment of free-ranging raccoon dogs (*Nyctereutes procyonoides*) infected with *Sarcoptes scabiei* in Kanagawa Prefecture, Japan

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Sarcoptes scabiei (*S. scabiei*) is an ectoparasite that infests humans as well as domestic and wild mammals. *S. scabiei* infections are considered a major cause of mortality among wildlife, because wild animals cannot be treated. In addition, an *S. scabiei* outbreak might dramatically decrease the wildlife population. In Japan, *S. scabiei* infection has spread among raccoon dogs (*Nyctereutes procyonoides*), which are a native species. In addition, this situation is a concern for humans as well as domestic and wild animals that inhabit the same geographic area. Accordingly, a measure to deal with the spread of *S. scabiei* infection by raccoon dogs must be developed to ensure a healthy ecosystem. Thus, it is important to understand the characteristics and the effect of *S. scabiei* infections in the wild. The present study revealed the following three points associated with *S. scabiei* infection in raccoon dogs.

In chapter 1, the epidemiology of raccoon dogs infected with *S. scabiei* and the influence of *S. scabiei* infections on the population of other Carnivora were revealed. Three zoological gardens in Yokohama city have engaged in rescuing sick or injured wildlife since 1972, and the records of raccoon dogs rescued between 1981 and 2010 were examined. The number of raccoon dogs rescued due to car accidents or other reasons increased from 1989 onwards. The records likewise revealed that an *S. scabiei* outbreak occurred in 1993, and that the infection spread from the southern to the northern regions of Yokohama. Further, the total number of raccoon dogs rescued peaked in 1995, but declined

thereafter. The high population density of the raccoon dog might have contributed to the radical spread of *S. scabiei* infection. Three Carnivora species, including raccoons (*Procyon lotor*), masked palm civets (*Paguma larvata*), and raccoon dogs inhabit Yokohama, and the habitats of these three species in urban areas are limited and partially overlap. In the Kanagawa prefecture, the raccoon is exterminated due to the alien species policy. Therefore, the raccoon population cannot be estimated in the present study. However, the masked palm civet and the raccoon dog are the targets of the wildlife rescue program, and their respective populations can be estimated. In the present study, the number of masked palm civets rescued gradually increased as the number of raccoon dogs rescued declined. Therefore, the raccoon dog and masked palm civet populations may be associated with each other in the wild. Consequently, the spread of *S. scabiei* in the raccoon dog might affect the population of the masked palm civet.

In chapter 2, it was revealed that debilitated raccoon dogs infected with *S. scabiei* exhibited abnormal hematological values compared to those of non-debilitated raccoon dogs infected with *S. scabiei*. Sarcoptic mange was considered a major cause of mortality. However, little is known about the hematological or serum biochemical values in clinically debilitated animals infected with *S. scabiei*. Accordingly, a comparison of hematological and serum biochemical values between severely debilitated and non-debilitated raccoon dogs infected with *S. scabiei* is presented in Chapter 2. The results indicated that the

white blood cell counts of both debilitated and non-debilitated raccoon dogs were increased. In addition, the total protein, albumin, glucose and calcium values of debilitated raccoon dogs were significantly lower than those of non-debilitated raccoon dogs. Conversely, the debilitated raccoon dogs had significantly higher aspartate aminotransferase, total bilirubin, blood urea nitrogen, and sodium, chloride and phosphorus values than the non-debilitated raccoon dogs. In particular, the increase in the blood urea nitrogen value was markedly dramatic. The results of the present study suggested that chronic infestations of *S. scabiei* debilitated raccoon dogs, and that the resultant hematological and serum biochemical abnormalities were caused by the infection.

In Chapter 3, effective treatments for *S. scabiei* infections in raccoon dogs were evaluated. Although the administration of ivermectin is the recommended treatment, many raccoon dogs in the Kanazawa Zoological Gardens have died due to *S. scabiei* infection, even after receiving a single ivermectin treatment. Since the hematological and serum biochemical abnormalities arose in raccoon dogs infected with *S. scabiei*, the effectiveness of treatment with an antibiotic for

all raccoon dogs and fluids for the debilitated raccoon dogs in conjunction with ivermectin administration was evaluated. As a result, the number of animals that survived was significantly greater after the administration of ivermectin along with an antibiotic for all raccoon dogs, as well as following the administration of fluid therapy to the debilitated raccoon dogs infected with *S. scabiei*. During the initial period, treatment to improve the general clinical condition was required prior to deworming treatment for *S. scabiei*.

In conclusion, the current study revealed the epidemiology of raccoon dogs infected with *S. scabiei* infection in Kanagawa Prefecture, Japan. Moreover, chronic infection of *S. scabiei* debilitated raccoon dogs, and caused hematological and serum biochemical abnormalities. Based on the results, an effective treatment to improve the survival rate of raccoon dogs infected with *S. scabiei* was established. The results provided valuable information to consider when evaluating *S. scabiei* infections in raccoon dogs, and contributed to the establishment of a methodology to ensure preservation of a healthy ecosystem.