PhD Defence Dao Thi Ha Thanh
Epidemiology of Opisthorchis spp. in Central Vietnam

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Summary
The Southeast Asian liver fluke Opisthorchis viverrini causes serious morbidity and mortality in the greater Mekong region. People infected with O. viverrini are at risk of developing cholangiocarcinoma. While O. viverrini is endemic in Central and South Vietnam, the epidemiology and the burden of disease that it causes have received little attention. Recently, our research group found an O. viverrini-like species in domestic ducks in a O. viverrini endemic area in Binh Dinh Province, Central Vietnam. This thesis aimed to study the occurrence and the life cycle of O. viverrini and the O. viverrini-like fluke in Central Vietnam. In the first chapter a general review of the literature of the Opisthorchis genus and opisthorchiasis is given, focusing on the morphology and the molecular biology, and on the epidemiology and the diagnosis of opisthorchiasis in SE Asia, including Vietnam. The chapter also gives a description on the finding of an Opisthorchis sp. in domestic ducks in Central Vietnam (further referred to as Opisthorchis sp. BD2013).

Following this chapter, the rationale and objectives of the thesis are given. The second chapter describes the current status of opisthorchiasis in a Central Vietnamese community. The apparent prevalence of O. viverrini in the population determined by stool examination was 11.4%. The mean number of worms recovered after treatment was 14.5. Male gender and the consumption of raw freshwater fish were found to be significant risk factors associated with opisthorchiasis in the area. In the third chapter, morphological and molecular identification was performed of an Opisthorchis sp. found in the bile ducts of domestic ducks in Binh Dinh Province. Morphological characteristics of the bird flukes were compatible with O. viverrini, although some characteristics differed from those described in specimens collected from mammal hosts. Computation of the phylogenetic trees on the partial sequences of ITS2 of the ribosomal (rb) DNA and COI markers of the mitochondrial (mt) DNA showed close similarity of Opisthorchis sp. BD2013 with O. viverrini. We speculated that these bird flukes were O. viverrini that showed intra-species morphological and molecular variability compared to isolates from mammals. In the fourth chapter, we provide new sequence data from the mitochondrial genome and the nuclear ribosomal transcription unit of Opisthorchis sp. BD2013. A phylogenetic analysis was conducted to clarify the basal taxonomic position of this species from ducks within the genus Opisthorchis. From four developmental life stages, the complete cytochrome b (cob), nicotinamide dehydrogenase subunit 1 (nad1) and cytochrome oxidase subunit 1 (cox1) genes; and near-complete 18S and partial 28S rb DNA sequences were obtained by PCR-coupled sequencing. Phylogenetic trees were inferred from concatenated (cob+nad1+cox1) nucleotide sequences and from combined 18S+28SrDNA nucleotide sequences of five Opisthorchis sp. BD2013 samples and additional reference taxa. Both trees demonstrated the anticipated clustering of taxa within the Opisthorchioidea, the paraphyly of the genus Opisthorchis and the sister-species relationship of Opisthorchis sp. BD2013 with O. viverrini. In the fifth chapter, we determined the infection rate and intensity of infection with Opisthorchis sp. BD2013 in ducks in 4 districts of Binh Dinh province. An infection rate of 34.3% was found; the intensity of infection was 13.8 worms per infected duck. The sixth chapter describes a study on the occurrence of O. viverrini and Opisthorchis sp. BD2013 in snails and fish intermediate hosts in the endemic area. A total of 12,000 snails belonging to six families, as well as 754 fish representing 12 species were examined. Shedding of O. viverrini cercariae was observed only in Bithynia s. goniomphalos and B. funiculata, at infection rates of 0.86% and 0.14%, respectively. O. viverrini metacercariae were found in 10 fish species. Carassius auratus, a fish species commonly
eaten raw, had the highest prevalence of 74.0%. Sharing of the same snail and fish intermediate host species was found for *O. viverrini* and *Opisthorchis* sp. BD2013. In the **seventh chapter**, the life cycle of *Opisthorchis* sp. BD2013 was developed. In the **eighth chapter**, we discuss the findings of our research on *Opisthorchis* sp. BD2013 in the context of its co-existence with *O. viverrini* in Central Vietnam and of the sharing of snail and fish intermediate hosts. There is a need to perform studies to define the host range of *Opisthorchis* sp. BD2013. Meanwhile, a One Health control program should be built for prevention and control of the neglected *O. viverrini* in the region.