Cryptosporidiosis is an important cause of diarrhea in humans and livestock, mainly in young and neonatal individuals. The effect of infection varies greatly, including mild to severe diarrhea, weight loss and dehydration, sometimes leading to mortality. Fasciolosis is an important parasitic disease of livestock, particularly of cattle, causing significant economic losses. Fasciolosis also occurs in humans, resulting in acute and chronic diseases of liver. Both cryptosporidiosis and fasciolosis cause negative social and economic impacts, especially in developing countries, including Vietnam.

The aim of this thesis is to obtain a greater understanding of the epidemiology of cryptosporidiosis and fasciolosis in livestock in Vietnam by: (i) estimating the prevalence of cryptosporidiosis and fasciolosis in common domestic animals in central Vietnam; (ii) identifying which Cryptosporidium species/genotypes and which Fasciola spp. harboring in domestic animals in central Vietnam; (iii) assessing the zoonotic importance of Cryptosporidium spp. and Fasciola spp., evaluating the current situation of cryptosporidiosis and fasciolosis in domestic animals in central Vietnam.

Chapter 1
Prevalence and molecular characterization of Cryptosporidium in native beef calves in central Vietnam

In this chapter, a cross-sectional survey was conducted, the aims of which were to investigate the prevalence of Cryptosporidium and to characterize the genotype distribution of Cryptosporidium isolates in native beef calves 2-6 months old in central Vietnam. The overall prevalence on the sample and herd levels were 18.9% (44/232) and 50% (20/40), respectively. Genotyping based on PCR and sequence analysis of the 18S rRNA gene revealed occurrence of the two non-zoonotic species Cryptosporidium ryanae and C. bovis, with the former as a dominant species in the animals. The absence of the zoonotic species C. parvum in calves examined suggests that the native beef calves 2-6 months old in the study area are unlikely to contribute to human cryptosporidiosis transmission.

Chapter 2
Prevalence, risk factors and molecular characterization of Cryptosporidium in pigs in central Vietnam

Part 1: Prevalence and risk factors associated with Cryptosporidium oocyst shedding in pigs in central Vietnam

In chapter 2, prevalence, risk factors and molecular characterization of Cryptosporidium in pigs in central Vietnam...
Vietnam were studied. In part 1, a cross-sectional survey was conducted. A total of 740 single fecal samples collected from diarrheic and non-diarrheic pigs on 89 farms were screened by the modified Ziehl-Neelsen staining method. Prevalence at the animal and the farm levels were 18.1% (134/740) and 71.9% (64/89), respectively. Risk factors for the infection were identified using univariate and multivariate logistic regression analysis. The results revealed that age, sanitary condition and topography were significantly associated with oocyst shedding \( (P<0.05) \). There was an association between the occurrence of diarrhea and the level of Cryptosporidium oocyst excretion within infected pigs.

This is the first epidemiological investigation of prevalence and risk factors of Cryptosporidium in pigs in Vietnam.

**Part 2: Molecular characterization of Cryptosporidium in pigs in central Vietnam**

In part 2, the genotype distribution of Cryptosporidium isolates in pigs in Vietnam was characterized. A total of 193 pig fecal samples were screened for the presence of Cryptosporidium oocysts using the modified Ziehl-Neelsen staining method, and 28 (overall prevalence 14.5%) were identified as positive by microscopic observation. Genetic identification based on the 18S ribosomal RNA and 70 kDa heat shock protein genes revealed that pigs in Vietnam are infected with two species/genotypes (Cryptosporidium suis and Cryptosporidium pig genotype II). The presence of these host-adapted species/genotypes suggests that pigs may not pose a significant public health risk in this area.

**Chapter 3**

Prevalence and molecular characterization of Cryptosporidium in ostriches (Struthio camelus) on a farm in central Vietnam

In chapter 3, prevalence and molecular characterization of Cryptosporidium in ostriches on a farm in Khanh Hoa province, central Vietnam were determined. A total of 464 ostrich fecal samples were examined Cryptosporidium oocysts, and 110 (overall prevalence 23.7%) were identified as positive by microscopy. Prevalence of Cryptosporidium in animals of <45 days, 45-60 days, 61-90 days, 91 days-12 months and >12 months was 23.5% (16/68), 33.3% (22/66), 35.2% (68/193), 0 and 5.8% (4/69), respectively \( (p <0.05) \). The majority of positive samples scored as the 3+ level of intensity of infection were from 61-90 days ostriches. Molecular analysis in the 18S ribosomal RNA, 70 kDa heat shock protein and actin genes demonstrated the presence of only Cryptosporidium avian genotype II in ostriches in central Vietnam.

**Chapter 4**

Prevalence and molecular identification of Fasciolosis in cattle in central Vietnam

Part 1: Prevalence of Fasciola in cattle and of its intermediate host Lymnaea snails in central Vietnam

In chapter 4, prevalence of Fasciola in cattle and of its intermediate host Lymnaea snails in central Vietnam was studied, and the genetic identification of Fasciola was described. In part 1, a cross-sectional study were conducted to investigate the prevalence of natural Fasciola infections in both the definitive hosts (cattle) and the intermediate hosts (Lymnaea snails) in central Vietnam. A total of 1075 fecal samples, randomly collected from cattle in Binh Dinh, Khanh Hoa and Phu Yen provinces, were examined for Fasciola eggs by a sedimentation method. The overall prevalence of Fasciola was 45.3%. A subset of the animals (235) were also screened for antibodies against Fasciola by an Enzyme-Linked Immuno-sorbent Assay. Overall, 46.3% of these animals were shedding Fasciola eggs while 87.2%
were *Fasciola* seropositive. A lower prevalence of *Fasciola* was observed in calves ≤ 2 years of age (37.6%) compared to that in cattle > 2 years of age (53.7%) (p < 0.05). The prevalence in the rainy season (50.8%) was significantly different to that in the dry season (38.1%) (p < 0.05). Of the 3,269 *Lymnaea viridis* and 1,128 *Lymnaea swinhoei* examined, 31 (0.95%) and 7 (0.62%), respectively, were found to be infected with *Fasciola*. This appears to be the first epidemiological survey of the prevalence of *Fasciola* in cattle and snails in central Vietnam, where human fasciolosis is hyperendemic in recent years.

**Part 2: Molecular identification of *Fasciola* spp. (Digenea: Platyhelminthes) in cattle from Vietnam**

In **part 2**, molecular characterization of *Fasciola* in cattle from central Vietnam was described. *Fasciola* spp. were collected from naturally infected cattle in Khanh Hoa province, Vietnam for morphological and genetic investigations. Microscopic examination detected no sperm cells in the seminal vesicles, suggesting a parthenogenetic reproduction of the flukes. Analyses of sequences from the first and second internal transcribed spacers (ITS1 and ITS2) of the ribosomal RNA revealed that 13 out of 16 isolates were of *Fasciola gigantica* type, whereas three isolates presented a hybrid sequence from *F. gigantica* and *Fasciola hepatica*. Interestingly, all the mitochondrial sequences (partial COI and NDI) were of *F. gigantica* type, suggesting that the maternal lineage of the hybrid form is from *F. gigantica*. No intra-sequence variation was detected. This result indicated that the genotypic variation also occurred in *Fasciola* population originated from central Vietnam.

The results of present thesis indicated that *Cryptosporidium* and *Fasciola* are highly prevalent in livestock in central Vietnam. For both *Cryptosporidium* and *Fasciola*, cattle is important reservoir for human and animal transmission. For *Cryptosporidium*, pigs and ostriches to a lesser extent are the reservoirs of some host-adapted genotypes. Due to *C. suis* and *Cryptosporidium* pig genotype II have been detected in some human cases, their findings give potential of infection from pigs to humans in central Vietnam. *C. parvum* has been detected in cattle in the same location previously, therefore, the role of cattle in disease transmission to humans should be paid attention. The role of other animal species, especially wildlife for *Cryptosporidium* and *Fasciola* are unclear and requires further studies. Control of *Cryptosporidium* and *Fasciola* might achieve through effective chemotherapy combined with good management strategy and good hygienic measures.