Comparison of the Prevalence of Liver Fluke Infection in Native Cattle and Native Swamp Buffaloes in Luang Prabang Province, Laos

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Abstract: This study aimed to survey the prevalence of *Fasciola* spp. infection in cattle and buffaloes in Luang Prabang province, Lao PDR (Laos). To determine the prevalence of *Fasciola* spp. a total of 100 individual fecal samples (50 from buffaloes and 50 from cattle) were collected from smallholder farms in seven locations within Luang Prabang province. The samples were examined in the lab using the Benedek Sedimentation method and Mc Master egg counting techniques. The results showed an apparent prevalence of *Fasciola* spp. infection of 12% across all herds, with 5 native swamp buffaloes (5%) and 7 native cattle (7%) infected. When comparing three age groups, it was discovered that 25% (5/20) of all buffaloes infected with *Fasciola* spp**.** were 1-3 years old, accounting for 10% of all buffaloes. Among the cattle, 7 infected individuals were 1-3 years old, representing 14% of the 50 cattle sampled. Specifically, 20% (3/15) of cattle aged 1-3 years were infected, 14.29% (2/14) of less than a year old were infected, and 9.52% of cattle older than three years were infected. The results indicate that the highest mean infection rate occurred in animals aged 1 to 3 years ($P < 0.05$). A comparison between species revealed no significant effect of species on the prevalence of *Fasciola* spp. ($P > 0.05$). Farmers in the seven villages raised large ruminants on a free-range basis in paddy rice fields post-harvest and on natural fodder and pasture grass. Therefore, further research on the comparative prevalence of *Fasciola* spp. infection in cattle and buffaloes across different seasons and farm systems is recommended.

Keywords: Fascioliasis, *Fasciola* spp., definitive host, parasitic disease, tropical ruminants, Lao PDR.

INTRODUCTION

Buffaloes and cattle are highly valuable ruminants for livestock production and have traditionally been used as draft animals. They are a crucial source of milk, meat, draught power, transportation, and on-farm manure in many Asian countries [1]. While buffaloes and cattle are well adapted to humid and high-temperature climates, cattle with high productivity are better suited to cooler climates. Furthermore, ruminants, especially buffaloes, are more efficient at utilizing feeds than cattle, particularly low-quality feeds [2]. According to FAOSTAT [3], the global buffalo population in 2022 was 205 million, representing a 4.36% increase since 2012. Meanwhile, the rapidly increasing human population and improvements in living conditions are driving greater demand for food, especially animal protein. Therefore, enhancing the potential of livestock production is essential, particularly in terms of animal health care and disease prevention. Parasitic disease prevention is a key factor in animal production and health, as it affects product quality and economic value [4].

Liver fluke (*Fasciola* spp.) is an important parasite in cattle and buffaloes, transmitted through food and water, which can occur from animals to humans [5, 6]. *Fasciola* spp. is commonly found in the tropics, such as in Africa and Asia [4]. Researchers have found outbreaks of liver flukes in ruminants all over the world. Tropical countries harbor *Fasciola* spp., a liver fluke that animals can contract by eating grass, and water plants harbor metacercaria, a contaminated parasite that causes Fasciolosis in Southeast Asia [4-7]. In Lao PDR, an ELISA diagnosis of liver flukes in local cattle revealed the presence of *Fasciola* spp. in 94.7% of cases [8]. *Fasciola* spp. is a significant parasite that not only negatively impacts the economy and animal production in Lao PDR but also poses a risk to human health. The reported prevalence of *Fasciola* spp. in cattle and buffaloes ranged from 0% to 85% [9]. Adult flukes live in the bile ducts and liver of the host. Hybridizing and laying eggs in feces that cause severe pathology occurs in the liver area, particularly in the liver tissue. This can lead to obstruction of the bile ducts, low growth, and reduced productivity, particularly in meat and milk when the animals are young or infected with parasites, potentially resulting in animal

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death, which is a cause of economic loss worldwide [4, 7]. However, the information on fascioliasis (liver fluke) infection in cattle and buffaloes in Luang Prabang Province, Laos, was limited. This study and survey hypothesized that liver fluke infection rates would be less than 50% in local ruminant livestock and that there would be no difference in infection rates between swamp buffalo and native cattle in Luang Prabang, Laos. Therefore, the main objective of this study was to determine the prevalence of fascioliasis infections in nativecattle and swamp buffaloes in Luang Prabang province, Laos.

MATERIALS AND METHODS

This study conducted a cross-sectional survey in Luang Prabang province, Laos, from April 20, 2023, to July 31, 2023 (summer to early rainy season). The aim was to detect the prevalence of fascioliasis (liver flukes) in native cattle (*Bos indicus Linnaeus*) and native swamp buffaloes (*Bubalus bubalis*) using fecal samples. Data were collected from fifty native cattle (1-3 years old, $n = 50$) and fifty native swamp buffalo (1-3 years old, $n = 50$), totaling 100 animals, seven locations in Luang Prabang: HuayloVillage, Hadkhang Village, Muang-KhaiVillage, Pha-Oh Village, Pakpa Village, NatanVillage, and DonemaiVillage (Figure **1** and Table **1**). The study adhered to all relevant guidelines and regulations and received approval from Souphanouvong University. Additionally, the ARRIVE guidelines were strictly followed throughout the entire study.

Data Collectionand Assessment of Infection Rates

The survey study was conducted by interviewing farmers who own cattle and buffaloes to understand how they prevent foliar parasite diseases in their animals raised in the pasture areas around the villages. Feces were collected directly from the anus of the animals using disposable long gloves between 6:00 and 7:00 a.m. before morning feeding (Figure **2**). The average sample size was about 30-40 g per replication, with 2 replications per animal. The samples were soaked in a sample tube containing a 10% formalin

Figure 1: Samples collectionat Pha-Oh village, Luang Prabang city.

Table 1: The *Fasciola* spp. Infection Rate of Ruminants in each Village

No	villages	Species	Sample, heads	Infection, heads	Infection rate, %
1	Huaylo	Buffalo	10	0	0.00
2	Hadkhang	Buffalo	19	3	15.79
3	Muang-Khai	Buffalo	11	0	0.00
4	Pha-Oh	Buffalo	10	2	20.00
5	Pakpa	Cattle	17	4	23.53
6	Natan	Cattle	16	3	18.75
7	Donemai	Cattle	17	0	0.00
Total			**100**	**12**	

Figure 2: Samples examination in the laboratory at the Faculty of Agriculture and ForestResource, Souphanouvong University, Laos.

solution, which was used to submerge the samples, and then placed in a plastic box to prevent the parasite eggs from changing shape.

The level of infection was determined using the Benedek Sedimentation method. Fecal parasite eggs were counted using the McMaster fecal egg counting procedure. The number of eggs in the chambers of two glass slides was calculated using the following formula:

Fecal parasite eggs (eggs/g) = (Total amount of eggs in a box of two glass plates × 60) / 40

The level of infection with liver fluke (*Fasciola* spp.) was classified as follows:

- Box with no eggs found: No infection (0)
- 1 - 100 eggs/g of feces: Low infection (+)
- 100 - 200 eggs/g of feces: Moderate infection level (++)
- More than 200 eggs/g of feces: Severe infection (+++)
- Times-Roman

Statistical Analysis

The collected data were encoded and recorded in an Excel database, then analyzed using descriptive statistics to determine the means and analyze the variance between the different datasets in this study. Pearson's chi-squared test was used for comparative statistical analysis using SPSS Statistics version 29, following the model: $\chi^2 = \sum \frac{(O-E)^2}{E}$ where **O** represents the observed value and **E** is the expected value.The statistical analysis showed a significant difference with P<0.05.

RESULTS AND DISCUSSION

The *Fasciola* spp. infection rate of ruminants in each villageis shown in Table **1**. All five infected buffaloes were from Pha-Oh (20%, 2/10) and Hadkhang village (15.79%, 3/19), while six infected cattle were from Pakpa (23.53%, 4/17) and Natan village (18.75%, 3/16). Furthermore, the study revealed that cattle younger than 1 year and older than 3 years had higher infection rates compared to buffaloes. Conversely, buffaloes aged 1 to 3 years had higher infection rates than cattle. When comparing the three

Table 2: Comparison by Age of Buffalo and Cattle Infected with *Fasciola* spp.

Age, years	Species	Sample, heads	Infection, heads	Infection rate, %	Mean rate, %	P-value
<1	Buffalo	6	0	0.00	10.00^{b}	0.040
	Cattle	14	2	14.29		
1 - 3	Buffalo	20	5	25.00	22.86^{a}	
	Cattle	15	3	20.00		
>3	Buffalo	24	0	0.00	4.44^{b}	
	Cattle	21	2	9.52		

Table 3: Prevalence of *Fasciola* spp. in Large Ruminants in Luang Prabang Province, Laos

Species	Sample, heads	Non-infection, heads	Infection, heads	Infection rate, %	P-value
Buffaloes	50	45	5	10	0.538
Cattle	50	47	7	14	
Total	**100**	**88**	**12**		

age groups, the study found that the highest mean infection rate occurred in animals (buffaloes and cattle) aged 1 to 3 years (22.86%; $P < 0.05$), as shown in Table **2**. Che-Kamaruddin *et al*. [10] identified age as a significant risk factor for bovine fasciolosis across all age groups, with older animals being 5.5 times more susceptible to infection in Taiping, Malaysia. This increased susceptibility is attributed to prolonged exposure to Fasciola-contaminated pastures in older animals [10]. During the sample collection for this study, younger animals (<1 year old) and older animals (>3 years old) were often kept in enclosures, while those aged 1 to 3 years were allowed to roam freely, leading to more frequent exposure to *Fasciola* spp. than the other groups. Shinggu *et al*. [11] suggested that when the prevalence of *Fasciola* spp. was not stratified by animal age, there was no significant association between age and fasciolosis, as animals of all ages were equally exposed to the infection depending on farm management practices. However, a comparison between species revealed no significant effect of species on the prevalence of *Fasciola* spp. ($P > 0.05$), as shown in Table **3**. This contradicts previous reports [12, 13], which demonstrated that the prevalence of fasciolosis in fecal and liver samples of buffaloes was higher than in cattle. This difference may be attributed to the season in which the survey was conducted, from summer to early rainy season. Elshraway and Mahmoud [13] reported that the seasonal prevalence rates of liver fascioliasis peaked in winter, decreased in spring and summer with no significant differences between buffalo and cattle, and then increased again after the rainy season, returning to a peak in winter. The rainy season provides fresh green grazing pastures, forest grazing, and riverside pastures with high-quality nutrition [14]. These conditions are suitable for the spread of *Fasciola* spp., as they serve as primary grazing areas [13, 14] and contribute to a higher prevalence of infection due to environmental conditions that favor the establishment and survival of the intermediate host [14, 15].

CONCLUSION

Based on this study, it can be concluded that animals aged 1 to 3 years had higher infection rates of *Fasciola* spp. Furthermore, there was no significant difference in *Fasciola* spp. infection rates between buffaloes and cattle during the summer season in Luang Prabang province, Laos. However, further in-depth research comparing the prevalence of *Fasciola* spp. infection between cattle and buffaloes across different seasons and farm systems is recommended.

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AUTHOR CONTRIBUTIONS

V. Chanthakhoun: Project administration, Conceptualization, Investigation, Methodology, Visualization; **V. Chanthakhoun, C. Suriyapha, S. Bounyavong, V. Vilaysack,** and **N. Manivanh**: Data curation, Formal analysis, and Software; **V. Chanthakhoun** and **M. Wanapat**: Resources, Supervision, Conceptualization, Validation, Visualization, Project administration and Funding acquisition; **C. Suriyapha**: Investigation, Validation, Visualization; **V. Chanthakhoun** and **C. Suriyapha**: Roles/Writing – original draft; **V. Chanthakhoun, S. Bounyavong, V. Vilaysack, N. Manivanh, C. Suriyapha** and **M. Wanapat**: Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

COMPETING INTERESTS

The authors declare no conflict of interest.

DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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