

## Prevalence of *Schistosoma japonicum* infection in water buffaloes in selected areas in Davao del Norte and Davao de Oro, the Philippines

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### Abstract

**Background and Aim:** Schistosomiasis (SCH) japonica is a zoonotic disease that can infect several mammalian hosts. Bovines, particularly water buffaloes, are considered the most important reservoir hosts due to the high levels of schistosome eggs, they excrete into the environment. In the Philippines, the previous studies have revealed a high prevalence of SCH in bovines in selected provinces in Luzon, Visayas, and Mindanao. However, there is a lack of studies on SCH in the Davao Region, Mindanao, where the infection remains a public health concern. This study aimed to determine the prevalence of SCH in water buffaloes in four barangays (villages) each in Braulio Dujali, Davao del Norte and Maragusan, Davao de Oro, Southeastern Mindanao, the Philippines.

**Materials and Methods:** Fecal samples from 91 water buffaloes were collected intrarectally, processed using the formalin-ethyl acetate sedimentation technique, and examined microscopically to determine the prevalence of SCH.

**Results:** A total of three water buffaloes (8.6%) in Maragusan were determined to be positive for *Schistosoma japonicum*, while none in Braulio Dujali. The prevalence of other parasites (*Fasciola* spp. [53.8%], *Paramphistome* spp. [20.9%], *Amphistome* spp. [16.5%], *Strongyle* spp. [11.0%], *Balantidium* spp. [9.9%], *Eurytrema* spp. [2.2%], and *Gongylonema pulchrum* [1.1%]) was also observed.

**Conclusion:** The results of the study provide baseline data on the presence of *S. japonicum* in water buffaloes in the Davao Region, Mindanao. Strategies for controlling and eliminating SCH in animal reservoir hosts, especially in water buffaloes, are necessary for the long-term control and elimination of SCH in humans.

**Keywords:** formalin-ethyl acetate sedimentation technique, prevalence, *Schistosoma japonicum*, schistosomiasis, water buffalo.

### Introduction

Schistosomiasis (SCH), which is caused by blood flukes from the *Schistosoma* genus, affects more than 230 million people worldwide [1]. It mostly affects poor and rural communities, mainly agricultural and fishing populations. *Schistosoma japonicum* is the causative agent of SCH in China, Indonesia, and the Philippines [2]. In the Philippines, SCH is endemic in 28 provinces across 12 regions, with approximately 12 million people at risk and 2.5 million directly exposed to it [3].

Unlike other schistosome species, SCH japonica is a zoonotic disease that can infect several mammalian

hosts, including cattle, water buffaloes, pigs, horses, goats, dogs, cats, rats, and monkeys [4-6], making it difficult to control the infection [6]. Bovines, particularly water buffaloes, are considered the most important reservoir hosts due to the high levels of schistosome eggs they excrete into the environment [7,8]. A study in China has shown that environmental contamination attributable to 238 infected bovines was approximately 28.7 million eggs per day [8].

Humans become infected with SCH when the larval forms of the parasite, released by the intermediate snail host *Oncomelania hupensis quadrasi*, penetrate the skin during contact with infested water. Provision of veterinary public health activities (which are defined as activities conducted at the human-animal interface that required to prevent, control, and eliminate suffering and economic loss caused by neglected zoonotic diseases such as SCH) is included in the recommended integrated approach by the World Health Organization to overcome the global impact of parasitic neglected tropical diseases [9].

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In the Philippines, recent studies on the prevalence of SCH in bovines were documented. In the Cagayan Province, 26% and 46% prevalence in water buffaloes were determined using enzyme-linked immunosorbent assay and polymerase chain reaction (PCR), respectively [10]. In the Leyte Province, high prevalence at 51.5% in water buffaloes was observed by the quantitative PCR (qPCR) technique [11]. Prevalence in bovines in the North Samar Province was also high at 62.09% and 81.07%, as determined using the formalin-ethyl acetate sedimentation (FEA-SD) and qPCR techniques, respectively [12]. A recent study in South Cotabato has shown that 48.6% of cattle and 60.5% of water buffaloes are positive for the infection as determined using the FEA-SD technique [13]. However, there are no studies documenting the presence of SCH in water buffaloes in the Davao Region, Mindanao, where SCH remains a public health concern.

Hence, this study aimed to determine the prevalence of SCH in water buffaloes in selected barangays (villages) in two provinces in Southeastern Mindanao, the Philippines.

## Materials and Methods

### Ethical approval

All animal handling procedures were approved by the Institutional Animal Care and Use Committee at the University of the Philippines (UP) Manila (UPM IACUC 2019-025). The study protocol was also approved by the UP Manila Research Ethics Board (UPMREB 2019-326-01). Informed consent was obtained from the owners before the collection of fecal samples, and confidentiality of data and privacy of water buffalo's owners were safeguarded.

### Study site, period, and sampling procedure

The municipalities of Braulio Dujali in Davao del Norte (DDN) and Maragusan in Davao de Oro (DDO), Southeastern Mindanao, the Philippines, were chosen as the study areas (Figure-1). Map was created using Free and Open Source QGIS software version 3.1.6. DDN comprises eight municipalities, three cities, and 223 barangays with a total population of 1,016,332, while DDO comprises 11 municipalities and 105 barangays, with a total population of 316,342 [14]. The main source of livelihood in both provinces is farming. A total of four barangays each in Braulio Dujali (Cabayanan, Magupising, New Casay, and Poblacion) and Maragusan (Lahi, Pamintaran, Tigbao, and Tupaz) were selected due to their known endemicity of SCH. The samples were collected in September 2019. The cross-sectional study utilized purposive sampling, wherein apparently healthy male and female water buffaloes in the SCH-endemic barangays were included in the study. Animals in the study site were normally seen tied to a tree and allowed to wallow in mudhole or irrigation canals during daytime and kept in a rural pen at night.

### Stool collection, processing, and examination

Fecal samples from 91 water buffaloes, aged between 7 months and 25 years old, were collected intrarectally by a team of veterinarians and animal technicians. Fecal samples (50 g for each animal) were weighed, labeled, and stored in a clean screw cap container with 10% formalin. The formalinized fecal samples were processed using the FEA-SD technique [15] and examined microscopically by three researchers. Two veterinary parasitologists validated observed parasites.

### Data processing and analysis

Data were encoded using Microsoft Excel 2016, processed, and analyzed using Epi Info version 7.2. (CDC, Atlanta, Georgia, USA). A sample was considered positive for a parasite if at least one ova/parasite was found in the microscopic examination. The prevalence of each parasite was calculated by dividing the number of positive cases by the total number of examined water buffaloes and multiplied by 100. To validate the identification, images of the parasites were examined by three microscopists and verified by two veterinary parasitologists from the Department of Veterinary Parasitology, College of Veterinary Medicine, University of the Philippines, Los Baños.

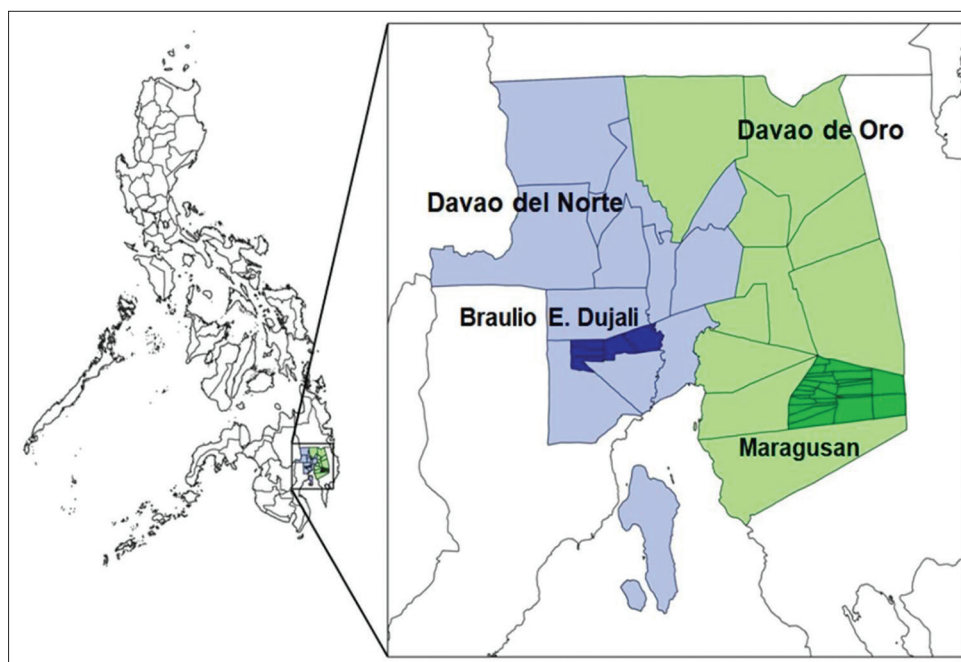
## Results

A total of 91 water buffaloes, 56 from Braulio Dujali and 35 from Maragusan, were included in the study. A total of 3 (8.6%) water buffaloes were determined to be positive for *S. japonicum* in Maragusan, while none in Braulio Dujali. *Fasciola* spp. was the most prevalent parasite (53.8%) observed in the sampled water buffaloes, followed by *Paramphistome* spp. (20.9%), *Amphistome* spp. (16.5%), *Strongyle* spp. (11.0%), *Balantidium* spp. (9.9%), *Eurytrema* spp. (2.2%), and *Gongylonema pulchrum* (1.1%) (Table-1). In Maragusan, one case of *Schistosoma* spp. was observed in a cow (Figure-2b). Representative images of the identified parasites are shown in Figure-2a-i.

## Discussion

This study investigated the presence of *S. japonicum* in water buffaloes in DDO, Southeastern Mindanao, the Philippines, using the FEA-SD technique. The overall prevalence (3.3%) of *S. japonicum* observed at the study sites was considerably lower than that in the previous studies, which used the same technique in West Samar (93.2%) [16], North Samar (62.1%) [12], and South Cotabato (60.5%) [13]. This discrepancy may be attributed to the low number of water buffaloes sampled due to the coincidence of stool collection period with the harvesting season in the study area. Sample turnout could be further improved by scheduling the collection on days where more animal owners are available.

In a study conducted in 2012, the FEA-SD technique provided a high sensitivity of 97.62% and was able to detect both high- and low-intensity



**Figure-1:** Map of the Philippines (left) and provinces of Davao del Norte and Davao de Oro (right) showing the selected municipalities [Source: Map generated using free and open-source QGIS software version 3.1.6].

**Table-1:** Prevalence of helminths in water buffaloes by formalin-ethyl acetate sedimentation technique in selected municipalities, Braulio Dujali, Davao del Norte and Maragusan, Davao de Oro, the Philippines, September 2019.

Parasite	Municipality				Total	
	Braulio Dujali (n=56)		Maragusan (n=35)		n=91	
	No.	(%)	No.	(%)	No.	(%)
<i>Fasciola</i> spp.	33	(58.9)	16	(45.7)	49	(53.8)
<i>Paramphistome</i> spp.	8	(14.3)	11	(31.4)	19	(20.9)
<i>Amphistome</i> spp.	10	(17.9)	5	(14.3)	15	(16.5)
<i>Strongyle</i> spp.	3	(5.4)	7	(20.0)	10	(11.0)
<i>Balantidium</i> spp.	8	(14.3)	1	(2.9)	9	(9.9)
<i>Schistosoma japonicum</i>	0	(0.0)	3	(8.6)	3	(3.3)
<i>Eurytrema</i> spp.	0	(0.0)	2	(5.7)	2	(2.2)
<i>Gongylonema pulchrum</i>	0	(0.0)	1	(2.9)	1	(1.1)

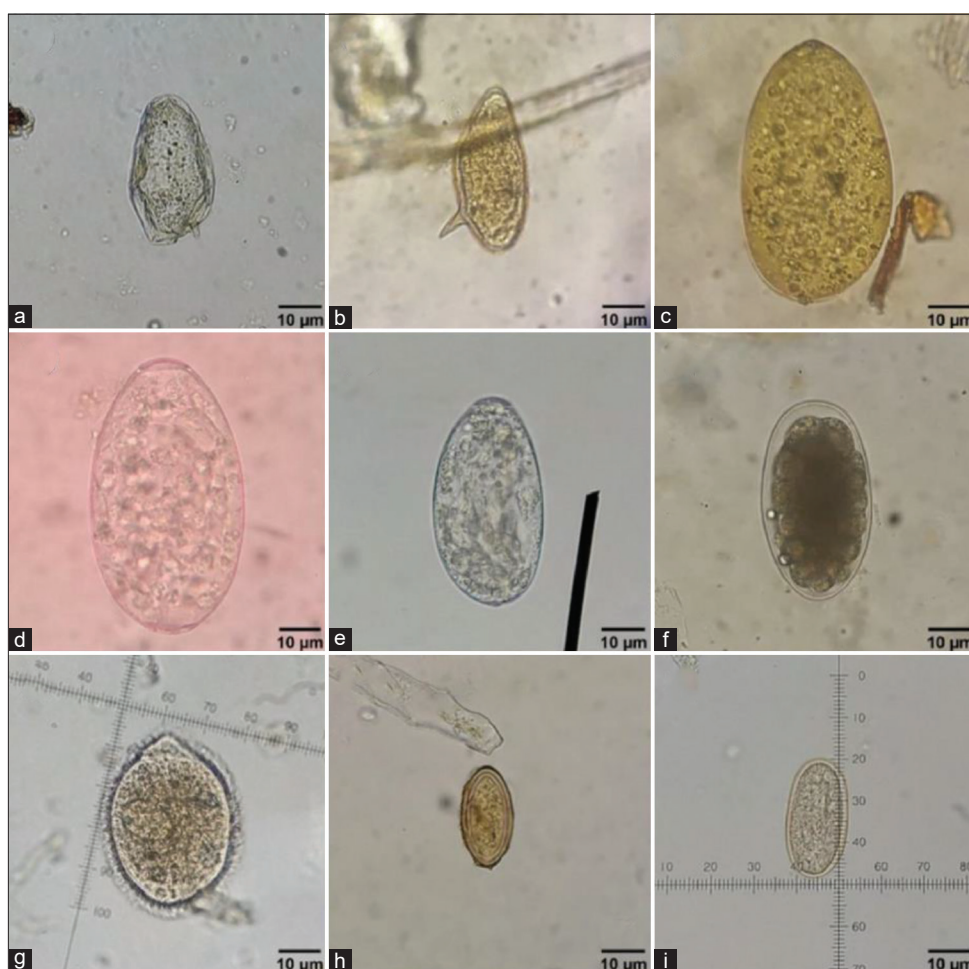
infections [16]. However, in 2015, FEA-SD was observed to have moderate sensitivity (60.8%) and low specificity (32.1%) with qPCR as a reference standard, which may have been due to lower fecal volume read [12]. The lower intensity of infection is harder to detect using less sensitive techniques [16]. Possible low-intensity infections could have been missed by the technique, which contributed to the low overall prevalence. This result can be improved by adjusting the FEA-SD protocol [16] so that all sediments after digestion are examined. Moreover, the use of a more sensitive technique (e.g., validated quantitative real-time PCR [qPCR] alone or in combination with FEA-SD) can provide a more accurate estimate of infections [12].

However, the low prevalence in water buffaloes in the Davao Region is similar to the results of the previous studies [17,18], where none or only a small proportion of water buffaloes were positive in an SCH-endemic region. In addition, water buffaloes tend to self-cure; thus, the time period they excrete

eggs in their feces is short [19]. This result suggests that even in endemic regions, infection in water buffaloes may vary. Surveillance among other mammalian hosts may be required to further understand the role of animals in the transmission of SCH in the region. The presence of snail intermediate host is also important in disease transmission. A previous study identified snail colonies in barangays in some municipalities in DDN [20] and DDO [21]. However, these snails may not harbor infectious cercariae at the time of collection, which may also contribute to the low prevalence of *S. japonicum* in water buffalo at one study site.

Despite the low prevalence observed, the presence of *S. japonicum* among water buffaloes can contribute to transmission and reinfection in both humans and water buffaloes, as determined in other studies [7,22]. DDN and DDO remain listed as provinces with high prevalence of SCH in humans [23]; thus, the role of water buffaloes needs to be fully established. Bovines, which act as reservoir hosts, may deposit their feces in water bodies near villages where humans





**Figure-2:** Parasite ova identified from water buffalo feces using formalin-ethyl acetate sedimentation technique. (a) *S. japonicum*. (b) *Schistosoma* spp. with long spine (in cow). (c) *Fasciola* spp. (d) *Paramphistome* spp. (e) *Amphistome* spp. (f) *Strongyle* spp. (g) *Balantidium* spp. (h) *Eurytrema* spp. (i) *Gongylonema pulchrum* (scale bar = 10 µm).

reside and go about their daily activities, which make cross-transmission inevitable [7]. The use of bovine feces as fertilizers in agricultural settings is another important factor in sustaining the disease cycle [24]. These conditions increase the risk factor of SCH zoonotic transmission and are most visible in areas (such as the study sites) where agriculture is the main source of livelihood, which compels farmers to rely on and live in proximity with water buffaloes. In addition, local villages tend to transport water buffaloes from SCH-endemic areas to non-endemic areas, which may facilitate the transmission of SCH. Comingling of water buffaloes with other livestock animals may also facilitate disease transmission [25]. Furthermore, the study provides baseline data on the infection in water buffaloes in Davao Region, Mindanao. Because the previous studies conducted in the country were limited to some areas in Luzon [10], Visayas [11,12,16], and South Cotabato, Mindanao [13].

A total of one stool sample obtained from a cow showed *Schistosoma* spp. ova with a prominent lateral spine (Figure-2b), which is a typical characteristic of *S. mansoni*. However, no cases of *S. mansoni* infection have been reported in the Philippines. *S. mansoni* is only reported in humans, monkeys, and

rodents [26,27], while bovines are known to be naturally infected [28]. This incidental result warrants an additional confirmation technique, such as PCR, to speciate and confirm identification.

*Fasciola* spp. was the most prevalent parasite (53.8%) observed in the sampled water buffaloes. A study conducted in North Samar also revealed the high prevalence of *Fasciola gigantica* in water buffaloes at 95.2% and 96.2% by FEA-SD and qPCR techniques, respectively. Furthermore, 56 water buffaloes were observed to be coinfecting with both *S. japonicum* and *F. gigantica* [12]. The high prevalence of *Fasciola* spp. has been observed in water buffaloes and is considered pathologic [29]. Two species, *F. gigantica* and *Fasciola hepatica*, are both important parasitic diseases affecting livestock and are known to cause human fasciolosis. Fasciolosis in humans has been reported in Asian countries such as Vietnam [30], Korea [31], and Nepal [32], but not in the Philippines. These findings raised public health importance and the need to survey both humans and animals in areas with high prevalence to investigate potential human infection. Molecular targets have been developed to speciate *Fasciola* spp. [33] and maybe useful tools to detect infection.

Other helminths were also observed in fecal samples despite the biannual deworming of water buffaloes. The current treatment regimen being used in DDN and DDO must be reviewed because albendazole, the drug of choice in the communities, is only effective against adult flukes [34]. A previous study in the Philippines also showed evidence of resistance of *Fasciola* spp. to albendazole, triclabendazole, and bromofenofos in water buffaloes [35]. This warrants another study to investigate anthelmintic susceptibility in bovines.

Effective prevention and control of SCH entails not only human but also animal and environmental health. SCH identified in the water buffaloes at the study sites can be used to support a One Health approach [3] and gather support from animal and health agencies, as well as other sectors toward control and elimination of SCH in the Philippines. Active surveillance and the use of appropriate techniques will aid in the accurate diagnosis of infection in animals. With the established presence of *Schistosoma* spp. among water buffaloes, strategies toward control and elimination of SCH in animal reservoir hosts are necessary for the long-term control and elimination of SCH in humans [36,37].

Treatment of water buffaloes with other drugs (e.g., praziquantel), as done in China [20], can be explored in the Philippines. Praziquantel has been determined to be 97% effective for SCH in water buffaloes without major side effects [20]. The data produced by this study can be used as a basis for the Department of Health (DOH) in including animal SCH in their control program. The DOH and Department of Agriculture are just beginning to strategize intervention measures to address the presence of animal reservoirs that facilitate transmission to humans [3]. Innovative approaches, such as shifting to mechanized farming, have also been shown to be effective [38]. Collaboration between these agencies is critical for coordinated efforts to cut the transmission of SCH through animals. The capacity building of regional animal disease diagnostic laboratories and local veterinarians is also needed to support the farmers in disease diagnosis and accurate reporting and to support disease control programs in the health sector.

## Conclusion

This study provides baseline data on the presence of *S. japonicum* in water buffaloes in selected barangays in the Davao Region, Mindanao, using the FEA-SD technique. Active surveillance in animals is necessary to estimate the disease's burden in animals and understand the role of water buffaloes in the transmission of *S. japonicum* in humans in the Philippines. Early detection and treatment in animals will prevent contamination of the environment and infection in humans.

## Authors' Contributions

RCN, AED, and VYB contributed to the conceptualization and design of the research. RCN, AED, and JMF performed literature search, data acquisition, data analysis, manuscript preparation, manuscript editing, and manuscript review. LADO performed literature search, data analysis, manuscript preparation, manuscript editing, and manuscript review. VYB helped in the manuscript preparation, editing, and review. All authors have read and approved the final manuscript.

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## Competing Interests

The authors declare that they have no competing interests.

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