Humoral responses to *Schistosoma japonicum* soluble egg antigens in domestic animals in Lindu Subdistrict, Central Sulawesi Province, Indonesia

Novericko Ginger Budiono[1], Sri Murtini[1,2], Fadjar Satrija[1,2], Yusuf Ridwan[1,2] and Ekowati Handharyani[3]

1. Parasitology and Medical Entomology Study Program, Graduate School, IPB University, Bogor, Indonesia;
2. Department of Animal Infectious Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia; 3. Department of Veterinary Clinics, Reproduction, and Pathology, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia.
Corresponding author: Fadjar Satrija, e-mail: fadjar_s@apps.ipb.ac.id
Co-authors: NGB: novericko_ginger@apps.ipb.ac.id, SM: srimurtini_fkh@apps.ipb.ac.id, YR: yridwan@apps.ipb.ac.id,
EH: ekowatieko@apps.ipb.ac.id
Received: 21-04-2020, **Accepted:** 22-06-2020, **Published online:** 29-08-2020

Abstract

Background and Aim: Schistosomiasis japonica, a disease caused by *Schistosoma japonicum*, is a public health problem in the Philippines, the Republic of Indonesia, and the People's Republic of China. The disease is known as zoonotic, meaning other than humans, animals are involved as the reservoirs. In Indonesia, schistosomiasis surveillance in animals is not continuous. Thus, the study to determine the prevalence of the disease in animals is needed. The study was aimed to determine the seroprevalence of *S. japonicum* infection among four species of domestic animals in the Lindu Sub-district, Central Sulawesi Province of Indonesia.

Materials and Methods: Blood samples of domestic animals were collected and analyzed for the presence of anti-*S. japonicum* immunoglobulin G antibodies against *S. japonicum* soluble egg antigens using the indirect hemagglutination assay. Animal stool samples were collected, and the miracidia-hatching assay was used for the detection of *S. japonicum* infection. Additional data concerning the animal identity and the management practices were obtained through a questionnaire used in surveys and interviews.

Results: A total of 146 sera from 13 cattle, 24 buffaloes, 54 pigs, and 55 dogs were collected. The overall schistosomiasis seroprevalence was 64.4%. The serology prevalence in cattle, buffalo, pig, and dog was 100.0%, 41.7%, 74.1%, and 56.4%, respectively. Domestic animals in all of five villages have previous exposure with *S. japonicum* as seropositive animals detected in every village. A total of 104 animal stool samples from 146 animals sampled were obtained. The overall schistosomiasis prevalence determined by the miracidia hatching assay was 16.35%. The sensitivity and specificity of indirect hemagglutination assay (IHA) in the current study were 88.24% and 41.37%, respectively, with miracidia hatching assay as the gold-standard method.

Conclusion: This study has shown a high seroprevalence of schistosomiasis japonica among domestic animals in the Lindu Subdistrict. IHA can be used as the screening method for the detection of *S. japonicum* infection in domestic animals. Chemotherapy and animal livestock grazing management programs to reduce the parasite burden and *Schistosoma* egg contamination in the environment must be implemented as part of one health approaches, in addition to other control measures.

Keywords: Indonesia, one health, schistosomiasis japonica, seroprevalence, zoonotic parasitic disease.

Introduction

Millions of people globally, especially those who are related to poverty, are affected by neglected tropical diseases. Schistosomiasis is one of the neglected tropical illnesses and a public health problem caused by the parasite from the genus *Schistosoma* [1-3]. The disease has global distribution as it spread in Asian, American, and African continents. There are seven species of *Schistosoma* identified as the etiological agents of schistosomiasis in humans, namely, *Schistosoma japonicum, Schistosoma mansoni, Schistosoma haematobium, Schistosoma mekongi, Schistosoma malayensis, Schistosoma guineensis*, and *Schistosoma intercalatum* [4-6]. Among these species, *S. japonicum* is the only species identified to be a true zoonotic parasite due to its capability to infect more than 40 species of mammals. In addition to its zoonotic nature, the complexity of the parasite's life cycle that involves the role of (snail) intermediate host makes the control measures hard to overcome [7].

Specifically, the disease caused by *S. japonicum* is known as schistosomiasis japonica [7]. Schistosomiasis japonica is a public health problem in the Republic of Indonesia, the People's Republic of China, and the Philippines [5]. The disease is known as zoonotic, meaning other than humans, animals are

involved as the reservoirs. In Indonesia, schistosomiasis surveillance in animals is not continuous. Thus, the study to determine the prevalence of the disease in animals is needed. Japan is another endemic country of the disease that declared eradication of schistosomiasis in 1996 as no new reported cases in 5 consecutive years. Japanese authorities included surveillance and intervention in humans as well as in animals in addition to substitution of the use of animal labors, snail surveillance, and intervention, and agro-engineering as part of the schistosomiasis eradication program [8]. One health approach by various parties would be better if it adapts to schistosomiasis control strategies at the local level with specific arrangements to improve public health interventions in countries where the disease is endemic [9].

The fact that a real interruption of transmission will require both domestic and wild animals that can support patent infections for the zoonotic species *S. japonicum* and *S. mekongi* is well recognized. The earlier studies were essential for the experimental study of schistosomes, while the latter studies concentrated on if any reservoir hosts could be transmitted to appropriate snails and thus contribute to human schistosomiasis. There is a need to consider if the animal is a reservoir and determine the active contribution of animal for human schistosomiasis transmission [10]. The complex biodiversity and transmission ecology of population groups between humans and other animals influences the success of control programs and increases elimination challenges. Indeed, not only should infection be removed in human populations to eradicate schistosomiasis but also block or minimize animal reservoir transmission [9,11]. However, the potential role of animal reservoirs in disease transmission and as an impediment to schistosomiasis elimination need to be further investigated [1,12-17].

S. japonicum infection spreads in the Philippines, Indonesia, and the People's Republic of China [5]. Central Sulawesi is the only endemic province in Indonesia, where the parasite infect humans and animals in 28 villages situated in three remote valleys, namely Lindu, Napu, and Bada [7]. Schistosomiasis is well known as a public health problem in the country and one of the priorities of disease eradication in both humans and animals. Unfortunately, routine surveillance of schistosomiasis in mammals other than humans only occurs in rodents. The presence of schistosomiasis japonica among domestic animals as well as wild animals other than rodents is not continuous. Thus, studies to identify the exposure rate of animals, especially domestic animals living around human beings, are needed.

Several researchers have conducted extensive studies in China and the Philippines regarding the transmission of the disease by the animals. In Indonesia, there are limited studies regarding infection by *S. japonicum* in animals due to scarce resources. With the aims of schistosomiasis eradication in 2025,

the Indonesian Government should consider the role of animals in the disease transmission. Additionally, the government can learn from the success of Japanese authorities implementing a multisector approach for schistosomiasis eradication.

To detect *S. japonicum* infection, the traditional tools are pathogen identification. First, the gold standard for the diagnosis of active schistosome infection is the discovery of eggs in a fecal smear using a light microscope and second, the miracidium hatching assay. Both these two methods have limitations, such as insensitive to detect low infection intensities, need more time, slightly dirty, require higher expenditure on labor, and time-consuming, and more involved procedures in the laboratory [18,19].

This study aimed to determine the seroprevalence of *S. japonicum* among four species of domestic animals in Lindu Subdistrict of the Sigi Regency, Central Sulawesi Province. To identify the rate of *S. japonicum* exposure in domestic animals, living in five endemic villages of the Lindu Subdistrict, indirect hemagglutination assay (IHA) was used and compared with Miracidial Hatching Assay (MHA) for detection of active schistosomiasis infection.

Materials and Methods

Ethical approval

Institute of Research and Community Service of IPB University approved the procedure of research with ethical approval No. 67/2017. The researchers asked the animal owner's permission for taking blood and stool samples from their animals. If the consent was collected, the animals were restrained, and the biodata was recorded.

Sera sampling of animals

A total of 146 serum samples from cattle, water buffaloes, dogs, and pigs were collected from five villages (Puroo, Langko, Anca, Tomado, and Olu) of the Lindu Subdistrict, Sigi District of Central Sulawesi Province in the second half of 2017. Animals that had never been treated with any anti-schistosome drugs were chosen randomly from the field. Types and numbers of animals examined in each village distributed according to Table-1. The blood (3 ml) obtained from each sampled animal (n=146) was placed in tubes without anticoagulant. The tubes were situated in a sloping position letting the blood clot to obtain serum. The tubes were centrifuged at 3.000 rpm for 10 min. Sera were collected and then stored at −20°C until further analysis. A total of 104 animal stool samples were obtained from 146 sampled animals. The stool samples were stored in a plastic clip and stored in the ice-box with 2-8°C and then stored in the refrigerator until the examination using the miracidia hatching assay.

IHA

In the current study, a commercial IHA kit was employed. In brief, 100 µl of normal saline was added into the first well of the transverse line, while 25 µl

Table-1: Seroprevalence of schistosomiasis among domestic animals in the Lindu Subdistrict.

Animal Species	Village											Total	
	Anca		Tomado		Langko		Puroo		Olu			Total	
	Positive	Prevalence (%)	Positive	Prevalence (%)	Positive	Prevalence (%)	Positive	Prevalence (%)	Positive	Prevalence (%)		Positive	Prevalence % (95% confidence interval)
Cattle	11/11	100.0	na	Na	Na	Na	na	na	2/2	100.0		13/13	100.0 (77.2-100.0)
Buffalo	na	na	6/7	85.7	4/17	23.5	na	na	na	na		10/24	41.7 (24.5-61.2)
Pig	12/16	75.0	0/1	0.0	12/12	100.0	11/16	68.75	5/9	55.6		40/54	74.1 (61.1-83.9)
Dog	10/10	100.0	6/6	100.0	5/20	25.0	1/5	20.0	9/14	64.3		31/55	56.4 (42.3-68.7)
Total	33/37	89.2	12/14	85.7	21/49	42.9	12/21	57.1	16/25	64.0		94/146	64.4 (56.3-71.7)

na=Not available

was put into the third and fourth well of the transverse line. Then, 25 µl of serum was added to the first well and mixed thoroughly. In a subsequent step, 25 µl of this mixed solution was added to the second well and mixed as before. Another 25 µl of mixed solution from the first well of the transverse line was also added to the third well and mixed. Then, 25 µl of the mixed solution in the third well was inserted to the fourth well and mixed. Therefore, the concentrations in the second, third, and fourth wells were 1:5, 1:10, and 1:20, respectively. In a simultaneous time, both positive and negative control sera were verified on each plate. One drop of 2.5% sensitized red blood cell was placed into each well, shaken, and then stored at room temperature for 1 h. Observations were made using the eye of researchers. The highest titer where agglutination still appeared was regarded as the terminal point of a positive reaction. If a positive reaction appeared at a titer ≥1:10, the serological test was considered to be positive. The seroprevalence is the number of seropositive animals over the total number of sampled animals. A village is defined as seropositive when at least one of its animals test positive to *S. japonicum*.

Miracidia hatching assay

Miracidia hatching assay was performed according to a previously published method [20,21] with some modifications. In the present study, the miracidia hatching assay was used as the gold-standard method for the detection of active schistosomiasis infection. Animal stool samples of about 10 g were first put into a plastic container added with water and homogenized with a stick. The homogenized samples were filtered using multilevel filters (the sizes of filters were 400, 100, and 40 µm). The retained mixture of fecal materials in the 40-µm filter was transferred into a triangular flask containing non-chlorinated water with a pH ranging from 6.8 to 7.6. The flask was left in a well-lit room with the temperature set at 24-30°C. The neck of the flask was strongly illuminated from one side and examined with a magnifying glass to detect the presence of free-swimming miracidia after 30 min, 1 h, 2, 4, 8, 12, and 24 h. The assay was stopped as soon as the hatching miracidium was determined. An animal was diagnosed as negative for infection if no miracidium could be seen after 24 h of observation.

Results

A total of 146 animal sera (13 cattle, 24 buffaloes, 54 pigs, and 55 dogs) were obtained (Table-1). The overall schistosomiasis seroprevalence was 64.4%. Schistosomiasis japonica seroprevalence was observed to be highest in cattle (100%), followed by pigs (74.1%), dogs (56.4%), and buffaloes (41.7%). The highest schistosomiasis prevalence at the village level was detected in Anca (89.2%), while Langko had the lowest seroprevalence (42.9%). The overall schistosomiasis prevalence determined by the

miracidia hatching assay was 16.35% of 104 fecal samples examined. The miracidia hatching assay was used as the gold-standard method for determining active schistosomiasis infection. The sensitivity and specificity of IHA in the current study were 88.24% and 41.37%, respectively (Table-2).

Discussion

One health as multidiscipline approach to a public health problem is better if it adapts to local schistosomiasis control strategies with specific arrangements to improve public health interventions in endemic countries [9]. Schistosomiasis japonica surveillance in Indonesia is limited, and there is no report of seroprevalence of *S. japonicum* among domestic animals in Indonesia. Our previous study identified the significant role of domestic animals, particularly buffalo and cattle, as main source of *S. japonicum* egg contamination to the environment [22]. Therefore, the control strategies of schistosomiasis japonica should include the control of animals as definitive hosts including drug intervention of the infected animals to reduce parasite burden and egg contamination [23,24]. Surveillance of animal schistosomiasis with reliable diagnostic techniques must become a part of the national surveillance program.

A novelty from this study is the first field study to report the use of IHA technique to detect the presence of antibody anti-*S. japonicum* soluble egg antigen (*SjSEA*) in domestic animals in Indonesia. Researchers previously reported high sensitivity and specificity the method in serological diagnosis of human schistosomiasis in the Philippines and the People's Republic of China [25-27].

The serology-based approach is one of the diagnostic methods for the detection of early pre-patent parasitic infection. Unfortunately, false positive as well as cross-reaction with other helminths can occur because these multicellular organisms are believed to have similar epitopes. In schistosomiasis endemic areas of China, IHA is currently the most frequently used immunodiagnostic assay for antibodies against schistosomiasis [28]. Other immunodiagnostic methods (DDIA and ELISA) are used for the detection of *Schistosoma* circulating antigens and the recognition of host antibodies against the parasite [29,30]. Nonetheless, immunodiagnosis is usually not species-specific. Furthermore, it may not offer effective

short-term cure detection [31,32]. The previous study found that settlers tested for schistosomiasis using IHA had high levels of a false-positive result [29]. The false-positive cases can be classified into three groups: (1) Infected hosts who were misdiagnosed with a stool test; (2) previously infected hosts who were healed after treatment; and (3) cross-reactions to antigenic epitopes of parasites other than *S. japonicum*. Furthermore, immunodiagnostic assays, in particular areas with high endemicity, have skipped some low-intensity infections [28].

Other than that, immunology approaches have more sensitivity than coprology ones. However, those methods have limitations, such as need a higher price and could present cross-reactivity with infections by other species of helminths. Notably, these tests might result in positive as the titer of antibodies in individuals given chemotherapy. The titer might not directly revert to negative after curing by chemotherapy. On the other hand, DNA-based detection technologies are highly sensitive and highly specific but are also very expensive and challenging on a large scale, especially in different field conditions. A more sensitive parasitological methodology used as an alternative is the hatching test for the detection of miracidia.

The serology-based test has two primary purposes: (1) To detect actual infections with clinical signs, and (2) to screen individuals without clinical signs but has the potency to be exposed with schistosomiasis-infested water bodies. Thus, a test with a high level of sensitivity is needed. In serology-based diagnosis, the known hindrance of active infection by the schistosomes is the extended seronegative window period. The production of antibodies in individuals with acute infection usually starts 4-7 weeks after the initial infection, even though most of the infected patients show seroconversion in 3-6 months [33].

In Indonesia, research studies focusing on the detection of *S. japonicum* antibodies in humans are scarce. These are limited to evaluation of the sensitivity and the specificity of enzyme-linked immunosorbent assay (ELISA) in the detection of *S. japonicum* excretory-secretory antigens in from infected people. Unfortunately, the study is only limited in a small scope (laboratory scope) [34]. The sensitivity and specificity of ELISA in detecting *S. japonicum* among humans were 74% and 78%, respectively, with Kato-Katz as the gold standard of diagnosis. Comparatively, the prevalence using the dot blot method and Kato-Katz was 24.27% (50/206) and 34.95% (72/206), respectively [35].

In the present study, the antigen used within the commercial kit is *S. japonicum* soluble egg antigen (*SjSEA*). Even though previous studies reported cross-reactivity of schistosome soluble egg antigen with antigens from nematodes and cestodes [36], other researchers found that false-positivity can be reduced by soluble egg antigen treatment using sodium meta periodate [37]. This finding showed that appropriate antigen preparation is necessary for diagnostics.

Table-2: The comparison of indirect hemagglutination assay with miracidia hatching assay for detecting schistosomiasis infection.

Diagnostic tool(s)	Miracidia hatching assay (gold standard)		
	Positive	Negative	Total
Indirect hemagglutination assay (screening test)			
Positive	15	51	66
Negative	2	36	38
Total	17	87	104

Animal surveillance in endemic sites that may delay the removal of this parasite disease is not generally performed for schistosomiasis [1]. For example, in Indonesia, animal schistosomiasis japonica surveillance in endemic sites is not sustainable. Only a limited report has been published. Schistosomiasis surveillance in animals other than humans has only routinely carried out in rodents. However, identifying animals contaminated with schistosomes, such as dogs, pigs, cattle, and buffaloes, need to be carried out, especially in Indonesia, where animal schistosomiasis surveillance is scarce. At present, available techniques must enhance the improved implementation of animal surveillance. One of the techniques is to establish serological tests using the available, and the widely used commercial kit is an IHA [28].

The present study faced several limitations, including (1) The limited number of samples taken, due to limited resources; (2) the present investigation was based on sampling from the limited participant in a limited region; and (3) it used *SjSEA* as an antigen target. The previous studies reported that the *SjSEA* antigen could cross-react with the other species of helminths [18,27,38-40]. The decline of antibody titers after schistosomiasis treatment is necessary for monitoring the effect of treatment [26]. Still, the coprology is the applied gold standard and also distinguishes species-specific characteristics [33].

Despite its limitations, IHA can be used in field conditions due to its simplicity and only includes simple laboratory equipment. Besides, the detection of schistosome infection using a serology-based test is still valuable, especially for humans who have travel history to endemic sites as well as new inhabitants such as immigrants [41]. Furthermore, an improved tool for the diagnosis of actual schistosome infection targeting circulating anodic antigen in serum can potentially be applied in the country for test-and-treat approaches [42]. To the authors' knowledge, the animals used in this study have never been treated with praziquantel. Thus, the seropositive individuals can be concluded as the ones that have the exposure of *S. japonicum* infection, even as previous or current infection. Serology-based schistosomiasis diagnosis based on the detection of antibody against *SjSEA* has adequate sensitivity but has limitation in the capability to distinguish between current active and past infections [26,43].

The current study introduced miracidia hatching assay for detection of *S. japonicum* among domestic animals in Indonesia. Even the method has not being applied to detect *S. japonicum* infection in humans in the country. To our best of the knowledge, serological detection for antibodies of *S. japonicum* in domestic animals is the first in Central Sulawesi. It was found that samples are positive to the *S. japonicum* antibody. The present study applied the first field trial of IHA as the detection tool of *S. japonicum* among domestic animals in the Lindu Subdistrict of Indonesia. The study also used miracidia hatching assay as the

complementary diagnostic test for the detection of *S. japonicum* infection. The Chinese government used miracidia hatching assay as a conventional method for detecting *S. japonicum* infection in both animals and humans in addition to IHA.

The results with IHA may reflect the prevalence at the population level, but IHA is not reliable at the individual level. Several causations for the inconsistent results of the ability of IHA to detect *S. japonicum* may be the following: (i) *SjSEA* is either crude or purified and from different kinds of hosts. The sensitized red blood cells are either from sheep or humans who are type "O" blood; (ii) the sensitivity of an examination method varies with the prevalence and intensity in a community; and (iii) positive reactions of IHA may continue for some time after chemotherapy or loss of infection, as for many immuno-diagnostic assays. However, finding positive results for individuals with past infection may be less of a problem, or even an advantage, in the identification of communities with infection (community diagnosis) [20]. In Indonesia, there is still no intervention such as chemotherapy used for domestic animals, so the prevalence of IHA here could reflect the prevalence in the population.

This study also compared two *S. japonicum* diagnostic techniques in domestic animals in Indonesia, namely, IHA and miracidia hatching assay. We conclude that the IHA is a better method for population screening of *S. japonicum* than the miracidia hatching assay. The miracidia hatching assay provides qualitative data useful for surveillance and is also most suitable for massive surveillance. The IHA kit used in this study detected the presence of immunoglobulin (Ig) G antibodies against *S. japonicum*. This IHA kit has been used extensively by other researchers, namely, in the study of which determines the seroprevalence of *S. japonicum* infection in China.

Therefore, further investigations and control strategies to reduce seroprevalence by doing sustainable surveillance are needed to elucidate the possible role of domestic animals in schistosomiasis transmission in the endemic sites. Among different serological methods, IHA assay as the screening test. Formerly, it has been reported that IgM antibodies may remain for an extended period up to several years after acute infection.

Furthermore, our study showed a high seroprevalence of schistosomiasis japonica in comparison to the previous coprology reports in the same region [22]. There is evidence that suggests, despite the constant presence of anti-*S. japonicum* IgM antibodies in serum, any positive IgM could not surely be considered as an acute infection. Thus, the coprology diagnosis to detect schistosome eggs or miracidia is required to declare individual active infection. Three significant challenges of schistosomiasis elimination are (1) low sensitivity of the most employed diagnostic tools, mainly where a low level of *S. japonicum* infection occur, and (2) diverse species of mammals

(including human) involve in the transmission of the disease, and the social and physical interrelationships between intermediate and definitive hosts [44].

The role of domestic animals in schistosomiasis transmission has never been further studied in the endemic sites in Indonesia due to limited resources. Furthermore, the surveillance programs of schistosomiasis among domestic animals as well as wild animals (except rodents) by the authorities are not continuous. Therefore, there is still a scarce of field data about animal schistosomiasis surveillance. According to Spear and Zhong [44], the lack of field data can lead to a lack of surveillance and control of information both practically and theoretically. This study can be additional information to both local and central government to make a comprehensive control program. Further studies must be carried out, such as the area where the infected free-range animals travel and the possible transmission sites.

Infection by *S. japonicum* is a pure zoonotic, different from infection by other *Schistosoma* species. *S. japonicum* naturally infected more than 40 mammalian species, for example, dogs, cats, goats, buffaloes, and pigs. The susceptibility of each species to the infection by *S. japonicum* was varied. For instance, goats, cattle, and rabbits are more susceptible to infection than rats, horses, pigs, and buffaloes [45,46]. Other studies have revealed that the worm recovery rate, egg production, and immunological response to the parasite of each host species were not similar [46]. For example, the schistosomes cannot mature or cannot cause considerable pathogenic changes in *Microtus fortis* [47].

The present study showed that exposure to *S. japonicum* was found in the four domestic animal species. In Lindu Subdistrict, cattle, buffaloes, and dogs, live freely in the intermediate host (*Oncomelania hupensis*) snail habitats. They may frequently contact with *S. japonicum* cercariae -infested water. The infected animals spread more eggs into the environment than human and other animal hosts [48]. The uninfected animals, such as water buffaloes, get an infection while they submerge those shelves in infected wallowing waters [49]. This study observed that calves graze in the fields together with their dams, instead of being stall-fed. Grazing calves increase the possibility of the calves acquiring the infection through exposure to the environment that is inhabited by the infective stage of the parasite. Furthermore, most of the pigs residing in the Lindu Subdistrict were kept in the cages. Pigs are mostly kept in cages or tethered in backyard of the houses. They possibly got infection due to contact with the *S. japonicum cercariae* contaminated water *used* for drinking water or washing the cages. Free roaming dogs often exposed to the infected snail habitat when the owners use the dogs for hunting or guarding crop fields.

The present study showed the usefulness of IHA assay for the detection of schistosomiasis among domestic animals. The assay can be scaled up in a broader scope of the country. The use of the assay for the detection of human infection by *S. japonicum* as the method can be used as a screening technique to simplify the labor-intensive of the coprology-based test.

The high seroprevalence of schistosomiasis japonica with IHA may occur due to the presence of false seropositivity. It means that it is possible for seropositive individuals classified into the negative group of fecal examination, especially in areas with relatively high endemicity. At the location of high endemicity, recurrent infections may occur even though chemotherapy has been administered [50]. Therefore, the IHA is well used as a screening test in a community but is not suitable for being used as a diagnostic test at the individual level. If each shows a positive result of *S. japonicum* in an antibody-based serological test, such as IHA, treated with praziquantel repeatedly, a large number of previous infections will be frequently administered. The improper treatment of uninfected individuals will result in inappropriate administration of praziquantel [50]. The IHA is the most commonly used screening test in the People's Republic of China to diagnose *S. japonicum* infection in humans [29,51,52] in addition to the miracidia hatching test [20].

The results of examining *S. japonicum* infection with the IHA technique reflect prevalence at the population level, but the results of the examination are not reliable at the individual level. The reason for the inconsistency of the ability of the IHA to detect the presence of *S. japonicum* infection can be due to several possibilities, for example, the circulating *S. japonicum* egg antigen (soluble egg antigen of *S. japonicum*) was acquired from several different species. Sensitized red blood cells also come from several different species, namely, from sheep or humans with blood type O. The IHA diagnostic kit used in this study uses blood type O human blood cells sensitized with soluble egg antigen from *S. japonicum*. There are variations in sensitivity at the different prevalence and intensity in a community. The results of positive IHA can continue for some time after chemotherapy or if the individuals examined have not been re-infected [20].

IHA sensitivity and specificity in diagnosing *S. japonicum* infection in this study were 88.24% and 41.37%, respectively. The sensitivity of the IHA of the study was slightly higher, and the specificity was relatively higher than that reported by Yu *et al.* [20], namely, 80.3% and 48.4%. Several other studies have shown that IHA sensitivity varied between 69.7% and 100%, with specificity between 35.7% and 93.6% [20,28,53-55]. Besides, there is the possibility of a cross-reaction with *Paragonimus westermani* of 64-84% with the *SjSEA* and 31.3% with *S. japonicum* purified egg antigen [50]. The concern in this study is that the sampled animals had never received

anti-*Schistosoma* therapy, so the results of seropositive without *S. japonicum* eggs or miracidia in the stool may be an active infection. Of particular concern is that when the host received anti-*Schistosoma* drugs, there is a possibility of false positives on the results of IHA. That is why the IHA is not used as a diagnostic technique for the individual level but can be used as a screening test [20].

If there is an anti-*Schistosoma* antibody in the host serum with an IHA technique, further testing is needed in the form of microscopic tests to detect the presence of eggs and miracidia of *S. japonicum* in the stool. Based on the World Health Organization recommendations, microscopic examination is the gold standard method in diagnosing *S. japonicum* infection. The purpose of this microscopic test is to determine whether the disease that occurs in the individual seropositive is an active infection (true positive) or not (false positive). For positive results in individuals who have had *S. japonicum* infection before, this test cannot be presumed. The advantages of the IHA technique in diagnosing schistosomiasis, which is this test, can be used to determine disease conditions at the community level (community diagnosis) [20].

In the Philippines, the surveillance of zoonotic schistosomiasis in animals is co-occurring with the buffalo anthelminthic mass treatment as well as with buffalo artificial insemination program. The concurring schistosomiasis surveillance with rabies vaccination in dogs has also been demonstrated [56]. Water buffaloes, as stated by Angeles *et al.* [56], are an excellent indicator of human infection by *S. japonicum*. The survey of schistosomiasis in water buffaloes needs to be involved in the guidelines for zoonotic schistosomiasis japonica. Without therapy, schistosomiasis is mostly a chronic disease in hosts that lasts for decades. It was caused by the high number of exposure and re-infection, which lead definitive hosts susceptible to infection [57]. A recent study reported that cattle and water buffaloes are the source of *S. japonicum* contamination in farming communities of Koronadal City, the Philippines. These species of animals played an essential role in schistosomiasis transmission [58].

Coproparasitology remains to be the gold standard. The detection of *S. japonicum* egg or miracidium from human or animal stool samples is being considered as the primary diagnostic method. Furthermore, as mentioned previously, serology-based diagnosis for schistosomiasis has limited capability to differentiate between the current and the previous infections [25,26,43]. In the current study, the authors used miracidia hatching assay as the comparison as well as the gold-standard method for the detection of active schistosomiasis infection [20]. The application of hatching assay in the detection of *S. japonicum* infection from negative eggs is primarily based on miracidia behavior, the strong positive phototropic [59]. The observation of the moving hatched

miracidia can be seen. The technique of miracidia hatching assay appeared to be inadequate for the detection of *S. japonicum* in the field condition, particularly for patients with low infection intensities [20].

The current study is a milestone concerning *S. japonicum* infection, as a public health problem, in Indonesian non-human mammals. Further researches focusing on parasitological detection, molecular detection, as well as environmental detection of schistosomiasis in Indonesia, are necessary. This study may encourage other researchers, as well as veterinary professions, to determine the burden of schistosomiasis in animals in the endemic areas in Indonesia. The World Health Organization guidelines well describe schistosomiasis surveillance and control programs. However, further assessment and observation need a standard tool by the World Health Organization for the validation of the elimination of schistosomiasis transmission [6]. The current study highlights the effectiveness as well as limitations of serology-based tests for detecting *S. japonicum* infection among domestic animals. Given the disease's zoonotic nature in Indonesia, it is evident that human mass drug administration alone will not control the incidence, prevalence, and morbidity of this disease [60].

Conclusion

Domestic animals in five villages of the Lindu Subdistrict have previous exposure to *S. japonicum* with seroprevalence of 64.4%. The IHA can be used as diagnostic screening tools for schistosomiasis in domestic animals despite its relatively high sensitivity (88.24%) and low specificity (41.37%). In addition to other control measures, new public and veterinary policy recommendations need to be implemented for controlling zoonotic schistosomiasis japonica in domestic animals in Indonesia, including chemotherapy and livestock grazing management to reduce parasite burden and contamination of *Schistosoma* eggs in the environment.

Authors' Contributions

Acknowledgments

Competing Interests

The authors declare that they have no competing interests.

Publisher's Note

Veterinary World (Publisher of International Journal of One Health) remains neutral with regard to jurisdictional claims in published institutional affiliation.

References

1. Angeles, J.M.M., Goto, Y., Kirinoki, M., Leonardo, L.R., Moendeg, K.J., Ybanez, A.P., Rivera, P.T., Villacorte, E.A., Inoue, N. Chigusa, Y. and Kawazu, S.I. (2019) Detection of canine *Schistosoma japonicum* infection using recombinant thioredoxin peroxidase-1 and tandem repeat proteins. *J. Vet. Med. Sci.*, 81(10): 1413-1418.

2. da Paixão Siqueira, L., Fontes, D.A.F., Aguilera, C.S.B., Timóteo, T.R.R., Ângelos, M.A., Silva, L.C.P., de Melo, C.G., Rolim, L.A., da Silva, R.M.F. and Neto, P.J.R. (2017) Schistosomiasis: Drugs used and treatment strategies. *Acta Trop.*, 176(2017): 179-187.

3. Fernandes, C.F., Pereira, S.D.S., Luiz, M.B., Zuliani, J.P., Furtado, G.P. and Stabeli, R.G. (2017) Camelid single-domain antibodies as an alternative to overcome challenges related to the prevention, detection, and control of neglected tropical diseases. *Front. Immunol.*, 8(2017): 653.

4. Lai, Y.S., Biedermann, P., Ekpo, U.F., Garba, A., Mathieu, E., Midzi, N., N'Goran, E.K., Raso, G., Assaré, R.K., Sacko, M., Schur, N., Talla, I., Tchuenté, L.A., Touré, S., Winkler, M.S., Utzinger, J. and Vounatsou, P. (2015) Spatial distribution of schistosomiasis and treatment needs in sub-Saharan Africa: A systematic review and geostatistical analysis. *Lancet Infect. Dis.*, 15(8): 927-940.

5. Gordon, C.A., Kurscheid, J., Williams, G.M., Clements, A.C., Li, Y., Zhou, X.N., Utzinger, J., McManus, D.P. and Gray, D.J. (2019) Asian schistosomiasis: Current status and prospects for control leading to elimination. *Trop. Med. Infect. Dis.*, 4(40): 1-29.

6. Zoni, A.C., Catalá, L. and Ault, S.K. (2016) Schistosomiasis prevalence and intensity of infection in Latin America and the Caribbean countries, 1942-2014: A systematic review in the context of a regional elimination goal. *PLoS Negl. Trop. Dis.*, 10(3): e0004493.

7. Satrija, F., Ridwan, Y., Jastal, J., Samarang, S. and Rauf, A. (2015) Current status of schistosomiasis in Indonesia. *Acta Trop.*, 141(1): 349-353.

8. Kajihara, N. and Hirayama, K. (2011) The war against a regional disease in Japan; A history of the eradication of Schistosomiasis japonica. *Trop. Med. Health*, 39(Suppl 1): 3-44.

9. Webster, J.P., Gower, C.M., Knowles, S.C.L., Molyneux, D.H. and Fenton, A. (2016) One health an ecological and evolutionary framework for tackling neglected zoonotic diseases. *Evol. Appl.*, 9(2): 313-333.

10. Colley, D.G. and Loker, E.S. (2018) New tools for old questions: How strictly human are "human schistosomes" and does it matter? *J. Infect. Dis.*, 218(3): 344-346.

11. Tchuem-Tchuenté, L.A., Rollinson, D., Stothard, J.R. and Molyneux, D. (2017) Moving from control to elimination of schistosomiasis in Sub-Saharan Africa: Time to change and adapt strategies. *Infect. Dis. Poverty*, 6(1): 1-14.

12. Matsumoto, J., Kirinoki, M., Kawai, S., Chigusa, Y., Ilagan, E.J., Ducusin, B.E., Yasuraoka, K. and Matsuda, H. (1999) Prevalence of schistosomiasis japonica among schoolchildren and animal reservoirs in Oriental Mindoro, Philippines. *Jpn. J. Trop. Med. Hyg.*, 27(2): 175-180.

13. Wu, H.W., Fu, Z.Q., Lu, K., Pond-Tor, S., Meng, R., Hong, Y., Chu, K., Li, H., Jiz, M., Liu, J.M. and Hou, M. (2017) Vaccination with recombinant paramyosin in Montanide ISA206 protects against *Schistosoma japonicum* infection in water buffalo. *Vaccine*, 35(26): 3409-3415.

14. Lu, D.B., Rudge, J.W., Wang, T.P., Donnelly, C.A., Fang, G.R. and Webster, J.P. (2010) Transmission of *Schistosoma japonicum* in marshland and hilly regions of China: Parasite population genetic and sibship structure. *PLoS Negl. Trop. Dis.*, 4(8): e781.

15. Macalanda, A.M.C., Angeles, J.M.M., Moendeg, K.J., Dang, A.T., Higuchi, L., Inoue, N., Xuan, X., Kirinoki, M., Chigusa, Y., Leonardo, L.R. and Villacorte, E.A. (2018) Evaluation of *Schistosoma japonicum* thioredoxin peroxidase-1 as a potential circulating antigen target for the diagnosis of Asian schistosomiasis. *J. Vet. Med. Sci.*, 80(1): 156-163.

16. Fernandez, T.J. Jr., Tarafder, M.R., Balolong, E. Jr., Joseph, L., Willingham Iii, A.L., Belisle, P., Webster, J.P., Olveda, R.M., McGarvey, S.T. and Carabin, H. (2007) Prevalence of *Schistosoma japonicum* infection among animals in fifty villages of Samar Province, the Philippines. *Vector Borne Zoonotic Dis.*, 7(2): 147-155.

17. Carabin, H., Balolong, E., Joseph, L., McGarvey, S.T., Johansen, M.V., Fernandez, T., Willingham, A.L. and Olveda, R. (2005) Estimating sensitivity and specificity of a faecal examination method for *Schistosoma japonicum* infection in cats, dogs, water buffaloes, pigs, and rats in Western Samar and Sorsogon Provinces, The Philippines. *Int. J. Parasitol.*, 35(14): 1517-1524.

18. Wen, L.Y., Chen, J.H., Ding, J.Z., Zhang, J.F., Lu, S.H., Yu, L.L., Shen, L.Y., Wu, G.L., Zhou, X.N. and Zheng, J. (2005) Evaluation on the applied value of the dot immunogold filtration assay (DIGFA) for rapid detection of anti-*Schistosoma japonicum* antibody. *Acta Trop.*, 96(2-3): 142-147.

19. Wilson, R.A. (2017) The problem with diagnosis of intestinal schistosomiasis. *EBioMedicine*, 25(2017): 16-17.

20. Yu, J.M., de Vlas, S.J., Jiang, Q.W. and Gryseels, B. (2007) Comparison of the Kato-Katz technique, hatching test and indirect hemagglutination assay (IHA) for the diagnosis of *Schistosoma japonicum* infection in China. *Parasitol. Int.*, 56(1): 45-49.

21. Li, H., Dong, G.D., Liu, J.M., Gao, J.X., Shi, Y.J., Zhang, Y.G., Jin, Y.M., Lu, K., Cheng, G.F. and Lin, J.J. (2015) Elimination of schistosomiasis japonica from formerly endemic areas in mountainous regions of southern China using a praziquantel regimen. *Vet. Parasitol.*, 208(3-4): 254-258.

22. Budiono, N.G., Satrija, F., Ridwan, Y., Handharyani, E., Murtini, S. (2019) The contribution of domestic animals to the transmission of schistosomiasis japonica in the Lindu Subdistrict of Central Sulawesi Province, Indonesia. *Vet. World,* 12(10): 1591-1598.

23. Cao, Z. Huang, Y., Wang, T. (2017) Schistosomiasis japonica control in domestic animals: Progress and experiences in China. *Front. Microbiol.* 8(2464): 1-5.

24. Roman, A.D., Gonzales, M.L.M., Amarillo, M.L., Belizario, V.Y. Jr., Liu, Q. and Alejandria, M.M. (2018) Therapeutic and prophylactic drug interventions for *Schistosomiasis japonicum*. *Cochrane Database Syst. Rev.*, 2018(6): CD010059.

25. Cai, P., Weerakoon, K.G., Mu, Y., Olveda, D.U., Piao, X., Liu, S., Olveda, R.M., Chen, Q., Ross, A.G. and

McManus, D.P. (2017) A parallel comparison of antigen candidates for development of an optimized serological diagnosis of schistosomiasis japonica in the Philippines. *EBioMedicine*, 24(2017): 237-246.

26. Hinz, R., Schwarz, N.G., Hahn, A. and Frickmann, H. (2017) Serological approaches for the diagnosis of schisto-somiasis a review. *Mol. Cell. Probes*, 31(2017): 2-21.

27. Xu, J., Peeling, R.W., Chen, J.X., Wu, X.H., Wu, Z.D., Wang, S.P., Feng, T., Chen, S.H., Li, H., Guo, J.G. and Zhou, X.N. (2011) Evaluation of immunoassays for the diagnosis of *Schistosoma japonicum* infection using archived sera. *PLoS Negl. Trop. Dis*., 5(1): e949.

28. Zhou, Y.B., Yang, M.X., Wang, Q.Z., Zhao, G.M., Wei, J.G., Peng, W.X. and Jiang, Q.W. (2007) Field com-parison of immunodiagnostic and parasitological tech-niques for the detection of schistosomiasis japonica in the People's Republic of China. *Am. J. Trop. Med. Hyg*., 76(6): 1138-1143.

29. Cai, Y.C., Xu, J.F., Steinmann, P., Chen, S.H., Chu, Y.H., Tian, L.G., Chen, M.X., Li, H., Lu, Y., Zhang, L.L. and Zhou, Y. (2014) Field comparison of circulating antibody assays versus circulating antigen assays for the detection of schistosomiasis japonica in endemic areas of China. *Parasit. Vectors*, 7(1): 138.

30. Feng, J., Xu, R., Zhang, X., Han, Y., He, C., Lu, C., Hong, Y., Lu, K., Li, H., Jin, Y., Lin, J. and Liu, J. (2017) A candidate recombinant antigen for diagnosis of schistosomiasis japon-ica in domestic animals. *Vet. Parasitol*., 243(1): 242-247.

31. Doenhoff, M.J., Chiodini, P.L. and Hamilton, J.V. (2004) Specific and sensitive diagnosis of schistosome infection: Can it be done with antibodies? *Trends Parasitol.*, 20(1): 35-39.

32. Duus, L.M., Christensen, A.V., Navntoft, D., Tarp, B., Nielsen, H.V. and Petersen, E. (2009) The *Schistosoma*-specific antibody response after treatment in non-immune travellers. *Scand. J. Infect. Dis*., 41(4): 285-290.

33. Kinkel, H.F., Dittrich, S., Bäumer, B. and Weitzel, T. (2012) Evaluation of eight serological tests for diagnosis of imported schistosomiasis. *Clin. Vaccine Immunol.*, 19(6): 948-953.

34. Samarang, S., Satrija, F., Murtini, S., Nurjana, M.A., Chadijah, S., Maksud, M. and Tolistiawaty, I. (2015) Detection of *Schistosoma japonicum* excretory-secretory antigens by ELISA method in human schistosomiasis in Napu Valey, Central Sulawesi. *Media Health Res. Dev.*, 25(1): 65-70.

35. Samarang, S., Nurjana, M.A., Maksud, M., Sumolang, P.P.F. and Gunawan, G. (2017) The diagnosis of schistosomiasis by dot blot method. *Media Health Res. Dev.*, 27(2): 99-104.

36. Chand, M., Chiodini, P. and Doenhoff, M. (2010) Development of a new assay for the diagnosis of schistoso-miasis, using cercarial antigens. *Trans. R. Soc. Trop. Med. Hyg*., 104(4): 255-258.

37. de Noya, B.A., Colmenares, C., Lanz, H., Caracciolo, M., Losada, S. and Noya, O. (2000) *Schistosoma mansoni*: Immunodiagnosis is improved by sodium metaperiodate which reduces cross-reactivity due to glycosylated epitopes of soluble egg antigen. *Exp. Parasitol.*, 95(2): 106-112.

38. Xu, X., Zhang, Y., Lin, D., Zhang, J., Xu, J., Liu, Y.M., Hu, F., Qing, X., Xia, C. and Pan, W. (2014) Serodiagnosis of *Schistosoma japonicum* infection: Genome-wide identi-fication of a protein marker, and assessment of its diagnos-tic validity in a field study in China. *Lancet Infect. Dis.*, 14(6): 489-497.

39. Yu, L.L., Ding, J.Z., Wen, L.Y., Lou, D., Yan, X.L., Lin, L.J., Lu, S.H., Lin, D.D. and Zhou, X.N. (2011) Development of a rapid dipstick with latex immunochromatographic assay (DLIA) for diagnosis of schistosomiasis japonica. *Parasit. Vectors*, 4(1): 157.

40. Deng, W., Xu, B., Hu, H., Li, J., Hu, W., Song, S., Feng, Z. and Fan, C. (2013) Diagnosis of schistosomiasis japon-ica with interfacial co-assembly-based multi-channel

41. electrochemical immunosensor arrays. *Sci. Rep.*, 3(2013): 1789.

41. Wilkins, P.P. and Keystone, J.S. (2013) Schistosomiasis serology is valuable and reliable. *Clin. Infect. Dis*., 56(2): 312-312.

42. van Grootveld, R., van Dam, G.J., de Dood, C., de Vries, J.J., Visser, L.G., Corstjens, P.L. and van Lieshout, L. (2018) Improved diagnosis of active *Schistosoma* infection in travellers and migrants using the ultra-sensitive in-house lateral flow test for detection of circulating anodic antigen (CAA) in serum. *Eur. J. Clin. Microbiol. Infect. Dis*., 37(9): 1709-1716.

43. Cai, P., Weerakoon, K.G., Mu, Y., Olveda, R.M., Ross, A.G., Olveda, D.U. and McManus, D.P. (2019) Comparison of Kato Katz, antibody-based ELISA and droplet digital PCR diagnosis of schistosomiasis japonica: Lessons learnt from a setting of low infection intensity. *PLoS Negl. Trop. Dis.*, 13(3): e0007228.

44. Spear, R., Zhong, B. and Liang, S. (2017) Low transmis-sion to elimination: Rural development as a key determi-nant of the end-game dynamics of *Schistosoma japonicum* in China. *Trop. Med. Infect. Dis*., 2(3): 35.

45. He, Y.X., Salafsky, B. and Ramaswamy, K. (2001) Host-parasite relationships of *Schistosoma japonicum* in mam-malian hosts. *Trends Parasitol*., 17(7): 320-324.

46. Zhai, Q., Fu, Z., Hong, Y., Yu, X., Han, Q., Lu, K., Li, H., Dou, X., Zhu, C., Liu, J. and Lin, J. (2018) iTRAQ-based comparative proteomic analysis of adult *Schistosoma japonicum* from water buffalo and yellow cattle. *Front. Microbiol*., 9(1): 99.

47. Jiang, W., Hong, Y., Peng, J., Fu, Z., Feng, X., Liu, J., Shi, Y. and Lin, J. (2010) Study on differences in the pathology, T cell subsets and gene expression in susceptible and non-sus-ceptible hosts infected with *Schistosoma japonicum*. *PLoS One*, 5(10): e13494.

48. Van Dorssen, C.F., Gordon, C.A., Li, Y., Williams, G.M., Wang, Y., Luo, Z., Gobert, G.N., You, H., McManus, D.P. and Gray, D.J. (2017) Rodents, goats and dogs-their poten-tial roles in the transmission of schistosomiasis in China. *Parasitology*, 144(12): 1633-1642.

49. Leonardo, L., Chigusa, Y., Kikuchi, M., Kato-Hayashi, N., Kawazu, S.I., Angeles, J.M., Fontanilla, I.K., Tabios, I.K., Moendeg, K., Goto, Y. and Fornillos, R.J. (2016) Schistosomiasis in the Philippines: Challenges and some successes in control. *Southeast Asian J. Trop. Med. Public Health*, 47(4): 651-666.

50. Zhou, Y.B., Zheng, H.M. and Jiang, Q.W. (2011) A diag-nostic challenge for schistosomiasis japonica in China: Consequences on praziquantel-based morbidity control. *Parasit. Vectors*, 4(1): 194.

51. Lv, C., Hong, Y., Fu, Z., Lu, K., Cao, X., Wang, T., Zhu, C., Li, H., Xu, R., Jia, B. and Han, Q. (2016) Evaluation of recombinant multi-epitope proteins for diagnosis of goat schistosomiasis by enzyme-linked immunosorbent assay. *Parasit. Vectors*, 9(1): 135.

52. van Dam, G.J., Xu, J., Bergquist, R., Claudia, J., Utzinger, J., Qin, Z.Q., Guan, W., Feng, T., Yu, X.L., Zhou, J. and Zheng, M. (2015) An ultra-sensitive assay targeting the circulating anodic antigen for the diagnosis of *Schistosoma japonicum* in a low-endemic area, People's Republic of China. *Acta Trop*., 141(1): 190-197.

53. Xu, J., Chen, N.G., Feng, T., Wang, E.M., Wu, X.H., Chen, H.G., Wang, T.P., Zhou, X.N. and Zheng, J. (2007) Effectiveness of routinely used assays for the diagnosis of schistosomiasis japonica in the field. *Chin. J. Parasitol. Parasit. Dis*., 25(3): 175-179.

54. Zhu, Y.C. (2005) Immunodiagnosis and its role in schistosomi-asis control in China: A review. *Acta Trop*., 96(2-3): 130-136.

55. Zhou, Y.B., Liang, S., Chen, G.X., Rea, C., He, Z.G., Zhang, Z.J., Wei, J.G., Zhao, G.M. and Jiang, Q.W. (2011) An integrated strategy for transmission control of *Schistosoma japonicum* in a marshland area of China:

Findings from a five-year longitudinal survey and mathematical modeling. *Am. J. Trop. Med. Hyg.*, 85(1): 83-88.

56. Angeles, J.M.M., Leonardo, L.R., Goto, Y., Kirinoki, M., Villacorte, E.A., Hakimi, H., Moendeg, K.J., Lee, S., Rivera, P.T., Inoue, N. and Chigusa, Y. (2015) Water buffalo as sentinel animals for schistosomiasis surveillance. *Bull. World Health Organ.*, 93(1): 511-512.

57. Colley, D.G. and Secor, W.E. (2014) Immunology of human schistosomiasis. *Parasite. Immunol.*, 36(8): 347-357.

58. Tenorio, J.C.B. and Molina, E.C. (2020) *Schistosoma japonicum* infections in cattle and water buffaloes of farming communities of Koronadal City, Philippines. *Int. J. One Health*, 6(1): 28-33.

59. Jurberg, A.D., Oliveira, Á.A.D., Lenzi, H.L. and Coelho, P.M.Z. (2008) A new miracidia hatching device for diagnosing schistosomiasis. *Mem. Inst. Oswaldo Cruz.*, 103(1): 112-114.

60. Olveda, R.M., Tallo, V., Olveda, D.U., Inobaya, M.T., Chau, T.N. and Ross, A.G. (2016) National survey data for zoonotic schistosomiasis in the Philippines grossly underestimates the true burden of disease within endemic zones: Implications for future control. *Int. J. Infect. Dis.*, 45(1): 13-17.

****