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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

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Schistosomiasis Transmission and Ecology in the Philippines (STEP) Project

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Abstract

Schistosoma japonicum causes a chronic parasitic disease, which persists as a major public health concern in The Philippines, the People's Republic of China and Indonesia. This infection is unique among helminthic zoonoses because it can infect humans and more than 40 other mammals. The objective of this study was to estimate the sensitivity and specificity of the Danish Bilharziasis Laboratory technique in cats, dogs, pigs, water buffaloes and rats in the Philippines. Faecal samples from each animal were collected on up to five occasions on five consecutive days in four villages of Sorsogon and Western Samar Provinces between January and July 2003. The faecal samples were analysed with the filtration and sedimentation Danish Bilharziasis Laboratory technique. Sensitivity and specificity of one, two, three, four, and five faecal samples were estimated using a Bayesian latent class approach. A total of 59, 43, 74, and 80% of the censored cats, dogs, pigs, and water buffaloes in the four villages were sampled, respectively. For all species, the sensitivity estimates when using the results of only 1 day of sampling were less than 80%. However, the sensitivity improved to at least 96% in all species when three or more faecal samples were collected on three separate days. The specificity was estimated to be above 92% across all species, even if just a single sample is used. The prevalences and 95% credible intervals of *S. japonicum,* adjusted for imperfect sensitivity and specificity, in cats, dogs, pigs, rats, and water buffaloes were 11.9% (6.8–18.3%), 19.9% (15.1–25.2%), 2.9% (1.1–5.2%), 31.3% (18.3–45.6%) and 6.3% (2.1–12.6%), respectively. Our results suggest that the Danish Bilharziasis Laboratory technique is valid for the detection of infection with *S. japonicum* in animals, and that sensitivity estimates are excellent when faecal samples are collected on at least three different days. Monitoring *S. japonicum* infection in animal reservoirs with a valid test could contribute to more effective public health control programmes.

Keywords: *Schistosoma japonicum*; Domestic animals; Rats; Sensitivity; Specificity; Diagnosis; Bayesian analysis; Philippines

1. Introduction

Schistosoma japonicum is a chronic parasitic disease, which persists as a major public health concern in parts of Indonesia (Kumar and Bubare, 1986), the Philippines and

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the People's Republic of China despite over 45 years of control efforts (McGarvey et al., 1999; He et al., 2001; Leonardo et al., 2002). This infection is unique among helminthic zoonoses in that the infection is transmitted naturally between man and more than 40 other mammals species (Nelson, 1975), a major obstacle for effective control (He et al., 2001). In the Philippines, it has been estimated that at least 6.7 million people live in endemic areas, 1.8 million of whom are considered to be directly exposed to schistosomes through water contact activities (Leonardo et al., 2002). Animals, including cattle, water buffaloes, pigs, goats, dogs and wild rats, have been found to play a role in contaminating the environment with *S. japonicum* eggs in endemic countries (Magath and Mathieson, 1945; Pesigan et al., 1958; Carney et al., 1978; Dumag et al., 1981; Fernandez et al., 1982; He et al., 1991; Wu et al., 1992; Zheng et al., 1997; Wang et al., 1998; Matsumoto et al., 1999). Most studies in the Philippines were conducted over 20 years ago with recent studies limited to the Provinces of Leyte and Mindoro (Matsumoto et al., 1999).

Several parasitological methods have been used to estimate the prevalence and intensity of infection with *S. japonicum* in animals, but, to our knowledge, there have been no studies reporting the sensitivity and specificity of coprology for the diagnosis of animal schistosomiasis japonica. The challenge with assessing the sensitivity and the specificity of diagnostic tests is that the gold standard for confirming the infection would involve sacrificing the animals, which is unethical for domestic animals. Another approach is to use statistical methods to estimate the sensitivity and specificity of diagnostic tests in the absence of a gold standard. In particular, latent class analyses from both frequentist (Walter and Irwig, 1988) and Bayesian (Joseph et al., 1995; Basáñez et al., 2004) viewpoints have been developed and often used in parasitology (Genser et al., 2001).

The objective of this work was to estimate the sensitivity and specificity of one, two, three, four or five consecutive days of stool samples, using the Danish Bilharziasis Laboratory (DBL) technique (Willingham et al., 1998) to detect infection with *S. japonicum* in cats, dogs, water buffaloes, cattle, pigs, and rats. The advantages of the DBL-technique are that it is a simple, quantitative and non-toxic technique that is able to distinguish live from dead eggs, to detect other helminthic species at the same time, can be stored before reading and can be reread for quality control. We also report the prevalences of infection in four endemic villages located in Western Samar and Sorsogon, the Philippines, adjusted for the estimated sensitivities and specificities of the test across one or more days.

2. Materials and methods

2.1. Locations

Four endemic villages within the provinces Western Samar and Sorsogon were selected for this study. These regions represent extremes in the use of man-made water management methods for irrigation of rice fields. The village of Buenavista (124° 1′43″E, 12° 41′19″N), in the municipality of Irosin, Sorsogon Province has a sophisticated irrigation system, all rice fields having access to water coming from two concrete dams, concrete main canals, or earth secondary canals. The village of Gumapia (123° 59′36″E, 12° 42′24″N), also in Irosin, is poorly irrigated with most rice areas depending on rainfall and a few having access to water from a temporary dam. The village of Bualo/Guindapunan (124° 51′15″E, 11° 58′54″N), in San Jorge municipality, Western Samar Province, is irrigated in most parts. The village of Pajo (124° 48′7″E, 11° 55′43″N), Tarangnan municipality, Western Samar Province, is a rain-fed community.

2.2. Study population

For livestock and domesticated animals, a door-to-door survey was conducted in all participating villages to establish a census. All animals meeting the inclusion criteria were sampled. Exclusion criteria included pregnancy, aggressiveness forbidding proper handling of the animals, animals being too young for rectal collection of faeces, refusal of the owner to agree to rectal collection of faeces from their animal or absence of the animal from the village at the time of sampling. Puppies and cats were kept in a cage for 5 days. Adult dogs, goats, cattle, water buffaloes (carabaos), and pigs were sampled every day for 5 days when possible. The sampling in the four villages took place between January 20, 2003 and July 18, 2003.

For rats, 25 rat traps were placed in the field at predetermined locations in each village using a geographic positioning system (GPS)-generated map as a guide. The traps were checked daily for the presence of rats. Cooked coconut meat or dried fish was used as bait. The traps containing the rats were brought to the field station for 5 days. All traps, which did not yield any rats after the third trapping night, were transferred to another location in the village.

2.3. Stool collection method

Rectal faeces collection was done daily for dogs, cattle, water buffaloes and pigs on five consecutive days. Efforts were made to collect at least 50–100 g of stool for each sample. Adult dogs were tied (using chain and collar) to ensure stool samples were collected the following day. Stools of rats, puppies, and cats were collected daily from their cages. Samples were placed in styrofoam boxes with freezer packs and at the end of each day, all samples were stored in a refrigerator. At the end of each week, all samples were transported to the Leyte State University Laboratory where they were analysed the following week. Appropriate codes were used to indicate what day during the previous week the samples were collected. Samples that were collected during the first day were processed first followed

by the samples collected the next day. Thus, there was a maximum of 7 days from the day of stool collection to the day of stool processing.

2.4. Parasitological methods

The filtration and sedimentation DBL-technique was used (Willingham et al., 1998). In brief, a 5 g sample from a homogenised 50–100 g faecal specimen was mixed with saline, shaken and passed through a series of sieves (400, 100, and 45 μm). The faecal material from the 45 μm sieve was washed into a sedimentation glass, filled with saline and left in the dark to sediment. The sediment was centrifuged and re-suspended with saline to obtain a volume of 2.25 ml and 150 μl of this solution was mixed with 850 μl 0.9% saline into 1 ml microscope chamber slides. Eggs were counted for three slides to obtain the total number of eggs per gram (epg) of faeces. If the number of eggs counted on one slide differed by 10% to the other readings, an additional slide was prepared and read to replace the count of the slide with a differing count. For samples smaller than the required 5 g, the epg was calculated by dividing total eggs counted in the three slides by the actual sample weight then multiplied by five. The egg counts read on 70 slides per reader were reviewed by one of the co-authors (EB or TF) for validation purposes.

2.5. Ethical issues

All research protocols with animals were approved by the Brown University Animal Care and Research Committee, the Research Institute for Tropical Medicine Research Ethics Committee and the DBL Ethics Committee. Captured rats were sacrificed as they have caused serious crop losses in rice fields, releasing of the animals after capture was not acceptable especially to the farming communities. The rats were euthanised with CO_2, which is an acceptable humane method for sacrificing rats.

2.6. Statistical analysis

We obtained between one and five stool samples from each animal selected for participation in the study. The sensitivity and the specificity of the DBL-technique are not 100% due to several biological and human factors that influence the outcome variable (epg). For example, the eggs of *Balantidium coli* can be confused with the eggs of *S. japonicum* in pigs, reducing the specificity of the technique. To take into account the imperfect nature of our diagnostic test, we used a variation of a statistical method discussed by Joseph et al. (1995). When more than one test result was available from a particular animal, we assumed that the results are independent from each other, conditional on that animal's true infection status. For example, if an animal is truly disease positive, the probability of having a positive test on day 1 is exactly the same as that on day 2, regardless of the result of the first test. Similarly, if an animal is truly negative, the probability of being found as a false positive on day 1 is exactly the same as that on day 2. The validity of our estimates depends on the validity of this assumption, which will be fully discussed later. The probability of any single test being positive within any animal is the sum of the probability of a true positive result and the probability of a false positive result. These are given, respectively, by the prevalence of the infection times the sensitivity of the test, and one minus the prevalence times one minus the specificity of the test. In mathematical terms, this implies $P = \pi \times S + (1 - \pi) \times (1 - C)$, where P is the probability of a positive test, π is the true prevalence of infection in the animal population under study, and S and C are the sensitivity and specificity of the test, respectively. Therefore, the unknown true infection status of the animal can be estimated once the sensitivity and specificity have been estimated. We will call this estimate the adjusted prevalence. When animals had more than one test, the probability P was used as the probability parameter of a binomial distribution. Our final model was formed by multiplying the binomial distributions obtained from each animal together. Separate analyses were carried out within each animal population, so that the adjusted prevalence, sensitivity and specificity were allowed to differ between different species. Following Joseph et al. (1995), we used a Bayesian approach for inferences. Uniform prior distributions on the range from 0 to 1 were used throughout, so that the final inferences were driven by the data rather than by any substantive prior information. Uniform priors imply that all values within their range, a priori, have the same probability of being the true value. As this model has no closed form solution, we used the Gibbs sampler to derive samples from the marginal posterior density for each parameter. Based on these samples, posterior medians were used as point estimates for adjusted infection prevalence, sensitivity and specificity, reported with 95% credible intervals (95% CrI) (credible intervals are the Bayesian analogue of standard confidence intervals). We also ran a similar model but with a hierarchical component to model the village-to-village variability in prevalence. As results are similar between the two models, we report only the results from the non-hierarchical model. WinBUGS software (Spiegelhalter et al., 2004) was used to implement the algorithm, with 10,000 iterations of the Gibbs sampler used for inferences. An initial 1000 iterations were used as burn-in and were discarded before parameter estimates are calculated. Model convergence was assessed using the Gelman–Rubin convergence statistic available in WinBugs (Spiegelhalter, D.J., Thomas, A., Best, N.G., 2004. WinBUGS version 1.4. (http://www.mrc-bsu.cam.ac.uk/bugs/)). A user-friendly program that implements these methods is available from http://www.medicine.mcgill.ca/epidemiology/Joseph/.

For comparison purposes, we also calculated the unadjusted prevalences in each species based on only the first stool sample and with any stool sample found positive.

To obtain the unadjusted prevalence with the first stool sample collected, we divided the number of animals with a positive sample at the first day of sampling by the total number of animals sampled. To obtain the unadjusted prevalence with any sample positive, we divided the number of animals with at least one positive stool sample by the total number of animals.

3. Results

Table 1 shows the numbers of animals censored and the distribution of the numbers of stool samples collected for each species per village. Eighty, 82, 63, 59, 43, and 74% of enumerated water buffaloes, cattle, cats, dogs, goats, and pigs, respectively, were sampled.

The numbers of goats and cattle sampled were too small to accurately estimate the sensitivity and specificity of the DBL-technique. In water buffaloes, cats, dogs, pigs and rats, the proportions of animals with at least three stool samples collected were 71, 53, 72, 96, and 98%, respectively (Table 1).

Table 2 shows the day-to-day variation in the DBL method results. In most species, the majority of animals that were found positive at least once did not remain positive all days that they were sampled. This table clearly shows that the conditional independence assumption is most likely met with our data.

Table 1
Number of animals enumerated, sampled and distribution of stool samples collected for each animal species in four villages of Sorsogon and Western Samar Provinces between January and July 2003

Village	Species						
	Water buffaloes	Cattle	Cats	Dogs	Goats	Pigs	Rats
Buenavista							
Number of animals enumerated	32	10	141	289	0	237	NA
Number of animals sampled	198	8	108	199	0	163	7
Number of animals with 1 stool sample	1	0	10	20	0	2	0
Number of animals with 2 stool samples	1	0	17	18	0	3	0
Number of animals with 3 stool samples	3	0	21	22	0	6	1
Number of animals with 4 stool samples	5	7	34	44	0	22	0
Number of animals with 5 stool samples	9	1	26	95	0	130	6
Gumapia							
Number of animals enumerated	11	0	94	154	12	90	NA
Number of animals sampled	7	0	61	86	4	71	1
Number of animals with 1 stool sample	0	0	12	16	0	1	0
Number of animals with 2 stool samples	0	0	24	16	0	0	0
Number of animals with 3 stool samples	1	0	21	23	0	2	0
Number of animals with 4 stool samples	2	0	4	26	4	44	0
Number of animals with 5 stool samples	4	0	0	5	0	24	1
Pajo							
Number of animals enumerated	26	0	56	37	0	56	NA
Number of animals sampled	29	0	35	27	0	41	25
Number of animals with 1 stool sample	0	0	11	3	0	0	0
Number of animals with 2 stool samples	3	0	15	3	0	1	0
Number of animals with 3 stool samples	1	0	7	5	0	2	0
Number of animals with 4 stool samples	8	0	2	11	0	11	2
Number of animals with 5 stool samples	17	0	0	5	0	27	23
Bulao/Guindapunan							
Number of animals enumerated	44	1	125	129	2	75	NA
Number of animals sampled	36	1	59	48	2	64	12
Number of animals with 1 stool sample	8	0	19	12	0	2	0
Number of animals with 2 stool samples	13	0	15	13	0	3	1
Number of animals with 3 stool samples	7	0	22	12	0	17	0
Number of animals with 4 stool samples	4	1	3	10	2	31	9
Number of animals with 5 stool samples	4	0	0	1	0	11	12
Total							
Number of animals enumerated	113	11	416	607	14	458	NA
Number of animals sampled	91	9	263	360	6	339	55
Number of animals with 1 stool sample	9	0	52	51	0	5	0
Number of animals with 2 stool samples	17	0	71	50	0	7	1
Number of animals with 3 stool samples	11	0	71	62	0	27	1
Number of animals with 4 stool samples	19	2	43	91	6	108	11
Number of animals with 5 stool samples	34	7	26	106	0	192	43

Table 2
Day-to-day variation in the results of the Danish Bilharziasis Laboratory method among animals that tested positive at least 1 day

Species	Water buffaloes	Cats	Dogs	Pigs	Rats
Number of negative animals without any change in status (%)	82 (90.1)	236 (89.7)	269 (74.7)	319 (94.1)	28 (50.9)
Number of positive animals without any change in status (%)	3 (3.3)	9 (3.4)	36 (10.0)	3 (0.9)	6 (10.9)
Number of animals with one change from negative to positive (%)	1 (1.1)	6 (2.3)	9 (2.5)	2 (0.6)	8 (14.6)
Number of animals with one change from positive to negative (%)	0 (0)	7 (2.7)	15 (4.2)	3 (0.9)	2 (3.6)
Number of animals with at least one change from positive to negative and one change from negative to positive (%)	5 (5.5)	15 (5.7)	31 (8.6)	11 (3.2)	11 (20.0)

Table 3
Median value (and 95% credible interval) of the sensitivity and specificity for 1, 2, 3, 4 or 5 stool samples collected in water buffaloes, cats, dogs, pigs, and rats sampled in Sorsogon and Western Samar Provinces between January and July 2003

		Species				
		Water buffaloes	Cats	Dogs	Pigs	Rats
Sensitivity	1 stool sample	78.0 (47.6–95.7)	65.0 (48.3–79.6)	75.0 (67.1–82.3)	77.1 (56.6–95.7)	76.8 (62.1–88.7)
	2 stool samples	95.2 (72.5–99.8)	87.7 (73.2–95.8)	93.8 (89.2–96.9)	94.8 (81.2–99.8)	94.6 (85.6–98.7)
	3 stool samples	98.9 (85.6–100)	95.7 (86.2–99.2)	98.4 (96.5–99.5)	98.8 (91.8–100)	98.7 (94.5–99.9)
	4 stool samples	99.8 (92.4–100)	98.5 (92.5–99.8)	99.6 (98.8–99.9)	99.7 (96.5–100)	99.7 (97.9–100)
	5 stool samples	100 (96.0–100)	99.5 (96.3–100)	100 (99.6–100)	99.9 (98.5–100)	100 (99.2–100)
Specificity	1 stool sample	98.7 (96.8–99.7)	97.2 (95.2–98.8)	97.0 (95.2–98.3)	99.1 (98.4–99.6)	92.6 (87.0–97.2)
	2 stool samples	97.4 (93.7–99.4)	94.4 (90.6–97.6)	94.1 (90.6–96.6)	98.2 (96.8–99.2)	85.7 (75.7–94.5)
	3 stool samples	96.2 (90.7–99.1)	91.8 (86.2– 96.4)	91.3 (86.3–95.0)	97.3 (95.2–98.8)	79.4 (65.9–91.8)
	4 stool samples	94.9 (87.8–98.8)	89.3 (82.1–95.3)	88.5 (82.1–93.4)	96.4 (93.8–98.4)	73.5 (57.3–89.3)
	5 stool samples	93.7 (85.0–98.5)	86.8 (78.2–94.1)	85.9 (78.2–91.8)	95.6 (92.3–98.0)	68.0 (49.8–86.8)

Table 3 provides the sensitivity and specificity estimates calculated for water buffaloes, cats, dogs, pigs, and rats. For all species, the sensitivity of only one stool sample was less than 80%. Collecting and analysing at least two stool samples improved the sensitivity considerably in all species, to 89% or greater. Conversely, and as expected, the specificity was reduced when increasing the number of stool samples analysed. Nonetheless, the specificity estimates remained at 85% or greater for cats, dogs, pigs, and water buffaloes with five stool samples. In rats, the specificity was quite low with one test and only reached a specificity of 68% (95% CrI: 49.8–86.8%) after five stool samples. There were no appreciable improvements in sensitivity for more than three stool samples. Similarly, the specificity estimates remained above 91% with three stool samples or less in cats, dogs, pigs and water buffaloes.

Table 4 displays the prevalence estimates, both adjusted and unadjusted for imperfect sensitivities and specificities that would be obtained if any positive stool analysis were considered as a positive result. It also provides the unadjusted results if only one stool sample had been collected per animal. The adjusted prevalences were highest in rats, followed by dogs and cats. The adjusted prevalences were very low in pigs and slightly higher in water buffaloes. No infected goats or cattle were found among the 15 animals tested. Table 4 also shows there was some variation

Table 4
Median value of the adjusted prevalence, prevalence with any sample positive and prevalence with only the first stool sample (and 95% credible interval) in water buffaloes, cats, dogs, pigs, and rats sampled in Sorsogon and Western Samar Provinces between April and June 2003

	Species				
	Water buffaloes	Cats	Dogs	Pigs	Rats
Adjusted prevalences					
Overall	6.3 (2.1–12.6)	11.9 (6.8–18.3)	19.9 (15.1–25.2)	2.9 (1.1–5.2)	31.3 (18.3–45.6)
Buenavista	1.5 (0.0–16.3)	17.6 (9.8–28.7)	14.9 (10.0–20.7)	0.5 (0.0–2.7)	32.9 (3.3–73.3)
Gumapia	0.0 (0.0–11.8)	6.5 (0.0–18.2)	20.2 (11.4–31.3)	0.0 (0.0–1.8)	13.3 (0.0–80.2)
Pajo	10.6 (2.4–27.3)	6.7 (0.0–23.9)	62.8 (41.8–81.9)	33.2 (17.4–50.9)	16.9 (5.0–35.3)
Bulao/Guidapunan	0.0 (0.0–5.5)	0.3 (0.0–6.0)	5.8 (0.6–16.8)	0.0 (0.0–1.7)	63.4 (30.1–91.1)
Overall unadjusted prevalence with any sample positive	9.9 (4.6–18.0)	14.1 (10.1–18.9)	25.3 (20.9–30.1)	5.9 (3.6–9.0)	49.1 (35.4–62.9)
Overall unadjusted prevalence with the first stool sample collected	3.3 (0.7–9.3)	9.5 (6.25–13.7)	16.1 (12.5–20.3)	2.7 (1.2–5.0)	21.8 (11.8–35.0)

in the adjusted prevalence estimates from village-to-village, even though most 95% CrI were quite wide because of small sample sizes in each village.

4. Discussion

To our knowledge, this is the first report of the performance of a coprological test in the diagnosis of animal *S. japonicum*. Our results show that the DBL-technique has a sensitivity of at least 96% when three stool samples or more are collected on three separate days in water buffaloes, cats, dogs, pigs, and rats. The DBL-technique had its lowest sensitivity in cats. This could be due to the difficulty in obtaining a sufficient amount of faeces from that species. The specificity was at least 91% when three stool samples or less were collected on three separate days in water buffaloes, cats, dogs, and pigs. The point estimate for the specificity in rats for three stool samples was 79%, but with very large credible intervals due to the small numbers of animals sampled. Results based on only one stool sample underestimate the true prevalence of animal infection, and are associated with sensitivity values of less than 80% across all species. This is very important for future public health monitoring systems of animal infection. These systems should use at least two or three stool samples collected on different days to increase the sensitivity to acceptable levels. The increase in sensitivity with increasing numbers of stool sample follows the principles governing heard sensitivity and specificity (Dohoo et al., 2003). The DBL-technique has a very high specificity in all species tested except in rats, where it was quite poor when more than three stool samples are used. If the prevalence is based on any positive stool examination, the true prevalence of the infection may be overestimated, when specificity is high. Low sensitivity of a single stool examination with the Kato–Katz has also been reported in humans (Engels et al., 1996; Yu et al., 1998; Booth et al., 2003).

Bayesian methods can be easily applied to estimate true prevalences in the absence of a gold standard test (Joseph et al., 1995; Basáñez et al., 2004), but our results are highly dependent on the assumption of conditional independence assumed by our model and difficult to verify in practice. We assumed that conditional on knowing the true infection status of the animal, the probability of an animal testing positive on day 1 is exactly the same as the probability of this animal testing positive on day 2, regardless of whether we know the result of the test on day 1 or not. Several arguments support the assumption of conditional independence here. First, the laboratory technicians were blinded as to which sample they were preparing and reading, which minimises information bias. For example, if an animal was a false positive on day 1 due to a slide that was not prepared correctly, it is unlikely that this would happen for the day 2 sample. Second, it has been shown, at least with human *S. mansoni*, that the shedding of eggs varies considerably from day to day. This has been documented and discussed at length in extensive work on this topic with *S. mansoni* in humans (de Vlas and Gryseels, 1992; Engels et al., 1996, 1997; de Vlas et al., 1997). It has also been observed with *S. japonicum* in humans (Aligui G.L., 1997. Quantifying human *Schistosoma japonicum* infection and exposure: errors in field diagnosis and uncertainties in exposure measurements. PhD Thesis, Brown University, RI, USA; Yu et al., 1998) and pigs infected experimentally (Giver et al., 2000). Third, it has been shown that eggs tend to cluster in the stool. Therefore, even though efforts were made to mix within each sample that was collected, we may not have collected all stool present in the rectum and thus increase the day-to-day variation (Ye et al., 1998). Fourth, we sampled animals on consecutive days. In this short time span, almost all variability in egg shedding will be random, as there is little time for conditions within the host or parasite to change. The above four reasons all demonstrate that within animals, which are truly positive, a variety of random factors can cause results to vary from day to day. In negative animals, as discussed above, almost all variations in test results will be due to random type errors, so that conditional independence almost certainly holds among animals that are truly negative. Finally, in the absence of a failsafe proof of conditional independence, our data suggest that this assumption may be met. In particular, we see much variability in test results within the same animal from day to day (Table 2). Over a 5-day period, many animals had changes in their test results, going from positive to negative and then back to positive, on consecutive days, and vice versa. If there were strong dependencies between the test results, we would have expected the diagnoses within the same animal to vary less from day to day. Furthermore, as several authors have discussed (Vacek, 1985; Dendukuri and Joseph, 2001; Gustafson, 2005), ignoring conditional dependence does not substantially change parameters estimates if the correlations given the true test status are small. Of course, our Bayesian model may not be appropriate for every data, and very large correlations can potentially bias parameter estimates.

The earliest record of non-human mammalian infection with *S. japonicum* was published in 1906 and since the 1940s several papers have reported infections in most mammal species (He et al., 2001). None of the following prevalence estimates, however, adjusted for imperfect sensitivities and specificities. In the Philippines, Dumag et al. (1981) found prevalences of 8.1, 4.3, 0.4, and 73.7% in dogs, pigs, water buffaloes, and rats, respectively, from samples collected in seven villages of Dagami pilot area in Leyte in 1979 and analysed with the merthiolate iodine formaldehyde concentration (MIFC) technique. Also in Leyte, a study conducted in the two municipalities of Mayorga and La Paz showed prevalences of 20.6, 14.9, 9.8, and 0% in dogs, pigs, water buffaloes, and cats, respectively, using the MIFC method combined with a circumoval

precipitin test (Fernandez et al., 1982). Only 19 cats were examined in that study which may not have been sufficient to detect a low prevalence of infection. These surveys were conducted before any large-scale treatment of humans with praziquantel had been implemented in the Philippines. Our results of adjusted prevalence of infection cannot be directly compared with these because we used different methods and these two studies were based on a single stool sample. Nonetheless, our results are in the range of what had been observed in the past. In addition, rats and dogs were shown to have the highest prevalence, which agrees with our results with adjusted prevalence. More recently (1997–1998), a study was conducted in two villages of the province of Mindoro Oriental on faeces from dogs and water buffaloes collected along the road. Prevalence of *S. japonicum* was estimated to be 13.1% [95% confidence interval (95% CI): 10.5–16.0%] in dogs (Matsumoto et al., 1999) whereas none of the 271 water buffaloes were found positive. These estimates may have been biased as some of the stool samples could have been from the same animal. In rats trapped by village volunteers, necropsied and microscopically examined for the presence of schistosomes in the mesenteric veins, a prevalence of 17.0% [95% CI: 14.2–20.2%] was found (Matsumoto et al., 1999). In comparing our results with those found in these previous studies it is important to realise that our adjusted prevalence values were higher than if the prevalence had been based on only one stool sample. This can be easily explained by the low sensitivity values when only one stool sample is collected. Conversely, the adjusted prevalence values were lower here than the prevalence based on any positive result. This is in part due to the fact that the specificity decreases with the number of stool samples and the sensitivity is very high for three samples or more.

Wild rats have been shown to be a host for *S. japonicum* in the Philippines (Pesigan et al., 1958; Jueoco, 1975. Studies on zoonoses associated with rodents. Report submitted to CCB-Tropmed Office as research fellowship grant from SEAMEO-TROPMED PROJECT; Cabrera, 1976; Oshima et al., 1978; Dumag et al., 1981; Matsumoto et al., 1999) and prevalences ranging from 22.7 to 86% have been reported from Leyte Province. In addition, Kamiya et al. (1980) examined the annual fluctuation of *S. japonicum* infection in rats in Leyte over 1 year and found a very high overall prevalence of 81.9% with the prevalence of infection increasing from February to April, maintained high until June after which it decreased.

One limitation of our study was that for some animals, especially dogs and cats, less than three stool samples could be obtained. This could be due to stress in cats when placed in cages. Further, dogs and cats in the villages were not fed regularly as this depends on the quantity of available left-over food at mealtime. Thus, defecation behaviour was also irregular. Dogs could be sampled more regularly than cats because intra-rectal collection of faeces could be done in adult dogs whereas the collectors had to wait for the cats to defecate in their cages. However, the sample sizes for dogs and cats were both reasonably large, and in general the credible intervals were narrow.

Some animals that had been censored in our study were not present in the village at the time of sampling. Some water buffaloes were used (rented) in another village during the scheduled sampling period. In other cases, the animals could not be caught (cats) or were too aggressive to be sampled (dogs), were pregnant (dogs and cats) or the owner refused to have their animals sampled. This could have affected our prevalence estimates if those animals that were not sampled were systematically more or less infected than those that were sampled. There is no reason to think that this was the case here and we therefore believe that our prevalence estimates are valid. It is important to note though that there was a very large variation in the prevalence estimates between villages.

Animal reservoirs may play an important role in human infection with *S. japonicum*. The infection is still endemic in the Philippines after several years of human control programmes (Leonardo et al., 2002). Monitoring infection in animal reservoirs with at least two stool samples collected on two consecutive days could contribute to more effective control programmes. Accurate determination of the intensity of infection in the mammalian host is also very important to the understanding and hence the modelling of the transmission dynamics of this infection (Anderson and May, 1991; Riley et al., 2005).

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