Document heading   doi: 10.1016/S1995−7645(14)60020−1

Survey on gastrointestinal parasites and detection of *Cryptosporidium* spp. on cattle in West Java, Indonesia

Sylvia Maharani Ananta[1], Suharno[1], Adi Hidayat[1], Makoto Matsubayashi[2*]

[1]*Parasitology Laboratory, Disease Investigation Center Subang, West Java, Indonesia. Directorate General of Livestock and Animal Health Services, Jl. Terusan Garuda Blok Werasari, RT.33/RW.11, Subang, West Java, Indonesia, 41212*
[2]*Laboratory of Protozoan Diseases, National Institute of Animal Health, National Agricultural and Food Research Organization, 3−1−5, Kannondai, Tsukuba, Ibaraki 305−0856, Japan*

ARTICLE INFO

ABSTRACT

Objective: To evaluate the presence of gastrointestinal parasites on cattle in Indonesia because the prevalence of parasites varies between countries depending on the terrain surrounding livestock farms and investigations in Indonesia have never been performed. **Methods:** Fecal samples from cattle at 35 farms in 7 districts in West Java, Indonesia, has been examined using the floatation or sedimentation methods, and a immunofluorescence assay and experimentally inoculation to mice for *Cryptosporidium* or *Giardia* spp. **Results:** 153 of 394 examined cattle (38.8%) were infected with gastrointestinal parasites. The prevalence of *Eimeria* spp., Nematoda spp. (including *Oesophagustomum* and *Bunostomum*−like), *Fasciola gigantica* and *Paramphistomum* spp. was 22.4%, 11.2%, 12.5% and 3.8%, respectively. *Cryptosporidium andersoni* (*C. andersoni*) was also found in two samples. One isolate of this parasite was confirmed to be transmitted to mice, in contrast to the isolates from other countries. **Conclusions:** although this survey is preliminary, the results shows that the infection of gastrointestinal parasites in Indonesia was not high, but these infected cattle could be as a potential source leading to economic losses in livestock production.

1. Introduction

A large number of gastrointestinal parasites, including Nematoda, Trematoda and Protozoa, often cause severe gastroenteritis in cattle worldwide. Infection by these parasites may affect the health status and productivity of cattle, particularly young cattle, which have a major impact, leading to considerable economic losses[1,2]. Each parasite has characteristic life cycles, *eg.*, some protozoan and nematode parasites become infective under ambient conditions, and some *Trematode* spp. require intermediate hosts and surroundings suitable for completion of their life cycle. In most cases, infection is transmitted by directly or indirectly by the fecal−oral route through environments contaminated with parasite eggs, oocysts or cysts. Since these organisms showed resistance to most environmental factors and chemical disinfectants, and could survive in long periods, the occurrence of infection is closely related to the animals′ surroundings[3]. Because hygienic treatments vary depending on local climate, farm management, animal nourishment conditions and strategic use of antiparasitic drugs, the prevalence of gastrointestinal parasites may vary between countries.

In 2010, according to the Statistical Book on Livestock, the population of cattle in Indonesia consists of 495 000 dairy cattle and 13 633 000 beef cattle. The management of animal production is mainly based on small commercial systems or family units in peri−urban and rural areas. The number of cattle per farm is not so large and on most farms is estimated to be less than 100 animals/farm. On

those production systems in Indonesia, only one study has been addressed the prevalence of *Fasciola gigantica* (*F. gigantica*)[4], but the occurrence of other gastrointestinal parasites, including heavy infection, has never been reported. Therefore, we firstly surveyed the infections in cattle to understand the presence of gastrointestinal parasites and subclinical infection as the potential source in Indonesia.

2. Materials and method

2.1. Samples

The investigation was conducted in West Java, Indonesia, from November 2011 to January 2012. Fecal samples were collected from 394 cattle without clinical symptoms at 35 farms in 7 districts. Those samples were taken from the rectum of each cow and put in separate plastic bag, then stored without any preservation at 4 ℃ until microscopic examination in the laboratory as described below.

2.2. Fecal examination

One gram of fecal samples was examined for intestinal protozoan oocysts and cysts, and helminth eggs using the sugar floatation method, as reported previously[5,6]. The interfaces of the sugar mixture were recovered after centrifugation, and were microscopically examined at 20× or 40×. When organisms like *Cryptosporidium* oocysts or *Giardia* cysts were detected, immunofluorescence assay (IFA) was performed to confirm the parasites using a commercial *Cryptosporidium*/*Giardia* detection kit according to the manufacturer's instructions (EasyStainTM; Biotechnology Frontiers, Australia).

For detection of trematode eggs, three grams of fecal samples were used for the sedimentation method, as reported previously[7]. Briefly, weighed feces were mixed in 250 mL of water in a measuring cup and filtered through a tea sieve. Filtrates were allowed to stand for more than 10 min to precipitate the eggs, then the supernatant was discarded. This step was repeated twice. Finally, the collected sediments were stained with 5% methylene blue and then observed under 20× or 40× magnification.

2.3. Experimental inoculation to mice

Cryptosporidium oocysts were purified from feces of cattle by sugar flotation method as described above, which yielded sufficient oocysts for experimental infection. Transmission to laboratory mice using the *Cryptosporidium* isolate was performed base on previous reported method[8]. Briefly, four

5–week–old female BALB/c mice (originally from Indonesian Research Center for Veterinary Science, Bogor, Indonesia) were orally inoculated with 1×10^6 oocysts in 0.1 mL of distilled water. Fecal samples were collected daily for 3 d post–inoculation, and then several times per week for 2 weeks post–inoculation. Those samples were examined for the presence of oocysts by sugar flotation method and count the number of oocysts per day (OPD), as described previously by Matsubayashi *et al*[9]. All animals were taken care based on the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Animal Health (Tsukuba, Ibaraki, Japan).

3. Results

3.1. Fecal examination

The results of fecal examination by the floatation and sedimentation methods are summarized in Table 1. Gastrointestinal parasites were observed in all districts, and 38.8% (153 of 394) of examined cattle were found to be infected with parasites. The most prevalent parasites, *Eimeria* spp., included *Eimeria bovis* (*E. bovis*) (oval shaped and approximately 28 μm× 20 μm in size) and *Eimeria zuernii* (*E. zuernii*)(spherical shaped and approximately 18 μm× 17 μm in size), were frequently detected (22.4%) and some cattle were infected with more than two species of *Eimeria* spp. The prevalence of Nematoda spp. was 11.2%, and species of each egg was morphologically estimated and thus described as species–like, because we could not identify them by the cultivation. Cysts, such as *Giardia duodenalis*, were not found, but *Cryptosporidium* spp. were detected in two cattle (Figure 1). Two *Cryptosporidium* isolates from cattle were morphologically the same, and ellipsoidal, measuring 7.2 μm × 5.4 μm and 7.2 μm× 5.3 μm. On IFA, these oocysts showed positive reactions for *Cryptosporidium andersoni* identification (Figure 1) and identified as *Cryptosporidium andersoni* (*C. andersoni*) based on these data. Smaller type oocysts such as *C. parvum*, which size are less than 6 μm, were not seen. Using sedimentation method for trematode eggs, *F. gigantica* and *Paramphistomum* spp. were found in 12.5% and 3.8% of samples, respectively. Prevalence varied by province; 0.0%–71.4% for *F. gigantica* and 0.0%–35.0% for *Paramphistomum* spp.

3.2. Transmission analysis of C. andersoni

Experimentally infection using one *C. andersoni* isolate on mice was conducted because we could obtain oocysts

Table 1

Prevalence of gastrointestinal parasitic infection in examined cattle stratified by district.

District (No. of farms)	No. of examined cattle	No. of cattle positive for parasites	Parasites detected by fecal examination				Parasites detected by sedimentation examination	
			Eimeria spp.	*C. andersoni*	Nematoda spp. (No. of positive cattle and species*)		*F. gigantica*	*Paramphistomum* spp.
Purwakarta (9)	22	14	6	0	2	(2; *Capillaria* spp., 1; *Trychostrongylus* spp.)	8	7
Tasikmalaya (15)	21	19	10	0	4	(1; *Bunostomum* spp., 1; *Cooperia* spp., 1; *Mecistocirrus* spp., 3; *Trychostrongylus* spp.)	15	4
Bogor (1)	183	67	47	1	22	(1; *Ascaris* spp., 1; *Bunostomum* spp., 1; *Cooperia* spp., 4; *Mecistocirrus* spp., 10; *Oesophagustomum* spp., 4; *Strongyloides* spp., 1; *Toxocara* spp., 6; *Trychostrongylus* spp.)	1	0
Majalengka (5)	22	4	2	0	0		2	0
Sumedang (2)	29	18	7	0	0		13	1
Kuningan (2)	13	2	2	1	1	(1; *Trichuris* spp.)	0	0
Ciamis (1)	104	29	14	0	15	(9; *Bunostomum* spp., 8; *Oesophagustomum* spp., 1; *Trichuris* spp., 5; *Trychostrongylus* spp.)	10	3
Total(%)	394	153(38.8%)	88 (22.4%)	2 (0.5%)	44 (11.2%)		49 (12.5%)	15 (3.8%)

*Eggs were classified based on egg morphology, and are thus described as species–like.

enough for this analysis from only one positive cattle. After inoculation, one of four mice shed oocysts at 4 d, and the number of oocysts per gram were less than 100. From days 7 to 14, large numbers of oocysts were found in feces, and the number of oocysts per gram reached at approximately 2× 10^4. During four–week monitoring period, the infected mice showed no clinical signs, including diarrhea, and oocysts were not detected from 18 d after inoculation.
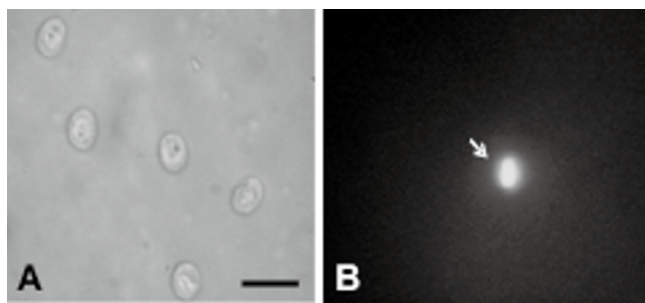
Figure 1. Picture of *C. anderson* detected in feces of a cow reared in Bogor, Indonesia.

(A) Immunofluorescence assay using a commercial *Cryptosporidium*/Giardia detection kit; (B) The arrow indicates positive reaction by an oocyst–specific antibody. Note: Scale bar = 10 μ m.

4. Discussion

In the present study, the prevalence of gastrointestinal parasites on cattle in Indonesia was found not to be high. Using the floatation method, *Eimeria* infection was found in 22.4% of examined cattle, with provincial levels ranging from 9.1%–47.6%. So far, the population of *Eimeria* spp. has varied among different countries in previous reports; 95.4% in Germany[10], 76.5% in Japan[6], 47.09% in Pakistan[11], 35% in Tanzania[12], and 33.33% in Brazil[13], although the key factors for *Eimeria* infection remain uncertain. In cattle, several *Eimeria* species, particularly *E. bovis* and *E. zuernii*, are known to induce clinical disease such as watery to bloody diarrhea[14]. Although species determination was not conducted in this study, oocysts with morphological similarities to *E. bovis* and *E. zuernii* were detected. Thus, these infected cattle could be as a potential source of bovine coccidiosis.

Nematode infections were found in most examined areas, and *Oesophagustomum* and *Bunostomum*–like eggs were frequently detected. However, the infection (11.2%) in the present study was relatively lower than other countries;

about 50% in Costa Rica and Vietnam[15,16]. Similar to oocysts of *Eimeria* spp., some *Oesophagustomum* spp. and *Bunostomum* spp. eggs form as an infective parasites after fecal excretion from the host, then remain on a favorable environment, which resulting in acquired infectivity. Since those eggs survive in soil or water for several weeks or months, infection by these parasites may provide insight into parasitic pollution and environmental hygiene conditions on farms. In West Java, small–scale farm management is common, and thus one possible reason for the low prevalence might be because of the low population of animals on each farm, leading to less opportunity to the transmission of infection.

The prevalence of Trematoda, particularly *F. gigantica* was found varied among the districts; it was between 0.0%–71.4%. This Trematoda cause significant economic losses, due to stunted growth, reproduction malfunction up to mortality. There has only been one report on the prevalence of Trematoda in cattle in Indonesia[4]. The report mentioned that based on egg counting examination, the infection rate of *F. gigantica* in Central Java was more than 40%. In Indonesia, this parasite is transmitted by snails namely Lymnaea rubiginosa[17], which act as an intermediate host for infection. The cattle were infected by ingestion of freshwater plants or rice straws containing the metacercariae of *F. gigantica*. Thus, the infection is closely associated with environmental factors, such as temperature, humidity and rainfall, which are suitable for intermediate hosts and parasites, as well as the chance of cattle feeding of aquatic plants. Based on these findings, the prevalence of infection could depend on the type of grazing. In the present survey, although little is known about the relationship between infection and farming method, cattle kept in cowsheds on smaller farms may have less opportunity to become infected.

In this survey, *C. andersoni* oocysts were detected in feces from two dairy cattle. In other countries, this *Cryptosporidium* species was also found in human[18,19]. Although little reports to clarify the pathogenicity of *C. andersoni*, it was suggested that chronic infection in cattle cause gastritis, reduces milk yield and leads to poor weight gain[20]. The infected cattle in the present study showed no clinical symptoms. To date, there have been no other reports about *Cryptosporidium* infection on livestock in Indonesia. Since the number of cattle examined in the present study was small, it is necessary to conduct further investigation in order to determine whether *Cryptosporidium* is more widespread in Indonesia.

On this study, *Cryptosporidium* isolate could be successfully transmitted to mice, although only one of four examined mice was infected. Previously, based on research done by Lindsay *et al*[21], the larger type oocysts of *Cryptosporidium* from cattle was distinguished from *Cryptosporidium muris (C. muris)* of rodents, and named as a new species on genetic and infectivity basis. However, *C. andersoni* isolates infective to mice, which had biological features different from those of other countries (not infective to mice), have been reported in two countries, Japan and Czech Republic[22–24]. Although it remains necessary to genetically analyze the isolate and to confirm infectivity using more mice or immunodeficient mice, this is the third report of *C. andersoni* isolates that could be infective to mice.

We firstly report about the prevalence of gastrointestinal parasites, *Eimeria* spp., *Cryptosporidium* spp., Nematoda spp. in cattle in Indonesia As a result of our current survey, the infection of gastrointestinal parasites was not high, and this preliminary survey could be used as a basic data on gastrointestinal parasitic infection in Indonesia. Most cattle farms in Indonesia are comparatively small scale, and animal hygiene treatment for infectious diseases varies among farmer. In this survey, the information about individual hygienic strategies on the examined farms, including anthelmintic treatment, could not be investigated. Thus, further extended survey in large areas in Indonesia is needed in order to evaluate the correlation between the use of anthelmintic treatment and prevalence.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

References

[1] Jiménez AE, Fernández A, Alfaro R, Dolz G, Vargas B, Epe C, et al. A cross–sectional survey of gastrointestinal parasites with dispersal stages in feces from Costa Rican dairy calves. *Vet Parasitol* 2010; **173**: 236–246.

[2] Perri AF, Mejía ME, Licoff N, Lazaro L, Miglierina M, Ornstein A, et al. Gastrointestinal parasites presence during the peripartum decreases total milk production in grazing dairy Holstein cows. *Vet Parasitol* 2011; **178**: 311–318.

[3] Kaewthamasorn M, Wongsamee S. A preliminary survey of gastrointestinal and haemoparasites of beef cattle in the tropical livestock farming system in Nan Province, northern Thailand. *Parasitol Res* 2006; **99**: 306–308.

[4] Estuningsih E, Spithill T, Raadsma H, Law R, Adiwinata G, Meeusen E, et al. Development and application of a fecal antigen diagnostic sandwich ELISA for estimating prevalence of *Fasciola gigantica* in cattle in central Java, Indonesia. *J Parasitol* 2009; **95**: 450–455.

[5] Matsubayashi M, Takami K, Kimata I, Nakanishi T, Tani H, Sasai K, et al. Survey of *Cryptosporidium* spp. and *Giardia* spp. infections in various animals at a zoo in Japan. *J Zoo Wildl Med* 2005; **36**: 331–335.

[6] Matsubayashi M, Kita T, Narushima T, Kimata I, Tani H, Sasai K, et al. Coprological survey of parasitic infections in pigs and cattle in slaughterhouse in Osaka, Japan. *J Vet Med Sci* 2009; **71**: 1079–1083.

[7] Charlier J, De Meulemeester L, Claerebout E, Williams D, Vercruysse J. Qualitative and quantitative evaluation of coprological and serological techniques for the diagnosis of fasciolosis in cattle. *Vet Parasitol* 2008; **153**: 44–51.

[8] Nagano S, Matsubayashi M, Kita T, Narushima T, Kimata I, Iseki M, et al. Detection of a mixed infection of a novel *Cryptosporidium andersoni* and its subgenotype in Japanese cattle. *Vet Parasitol* 2007; **149**: 213–218.

[9] Matsubayashi M, Kimata I, Iseki M, Hajiri T, Tani H, Sasai K, et al. Infectivity of a novel type of *Cryptosporidium andersoni* to laboratory mice. *Vet Parasitol* 2005; **129**: 165–168.

[10] Bangoura B, Mundt HC., Schmäschke R, Westphal B, Daugschies A. Prevalence of *Eimeria bovis* and *Eimeria zuernii* in German cattle herds and factors influencing oocyst excretion. *Parasitol Res* 2012; **110**: 875–881.

[11] Rehman TU, Khan MN, Sajid MS, Abbas RZ, Arshad M, Iqbal Z, et al. Epidemiology of *Eimeria* and associated risk factors in cattle of district Toba Tek Singh, Pakistan. *Parasitol Res* 2011; **108**: 1171–1177.

[12] Chibunda RT, Muhairwa AP, Kambarage DM, Mtambo MM, Kusiluka LJ, Kazwala RR. Eimeriosis in dairy cattle farms in Morogoro municipality of Tanzania. *Prev Vet Med* 1997; **31**: 191–197.

[13] Almeida Vdos A, de Magalhães VC, Neta Ede S, Munhoz AD. Frequency of species of the genus *Eimeria* in naturally infected cattle in Southern Bahia, Northeast Brazil. *Rev Bras Parasitol Vet* 2011; **20**: 78–81.

[14] Daugschies A, Najdrowski M. Eimeriosis in cattle: current understanding. *J Vet Med B Infect Dis Vet Public Health* 2005; **52**: 417–427.

[15] Jiménez AE, Montenegro VM, Hernández J, Dolz G, Maranda L, Galindo J, et al. Dynamics of infections with gastrointestinal parasites and *Dictyocaulus viviparus* in dairy and beef cattle from Costa Rica. *Vet Parasitol* 2007; **148**: 262–271.

[16] Geurden T, Somers R, Thanh NT, Vien LV, Nga VT, Giang HH, et al. Parasitic infections in dairy cattle around Hanoi, northern Vietnam. *Vet Parasitol* 2008; **153**: 384–388.

[17] Suhardono, Roberts JA, Copeman DB. Biological control of *Fasciola gigantica* with *Echinostoma revolutum*. *Vet Parasitol* 2006; **140**: 166–170.

[18] Leoni F, Amar C, Nichols G, Pedraza–Díaz S, McLauchlin J. Genetic analysis of *Cryptosporidium* from 2414 humans with diarrhoea in England between 1985 and 2000. *J Med Microbiol* 2006; **55**: 703–707.

[19] Morse TD, Nichols RA, Grimason AM, Campbell BM, Tembo KC, Smith HV. Incidence of cryptosporidiosis species in paediatric patients in Malawi. *Epidemiol Infect* 2007; **135**: 1307–1315.

[20] Anderson BC. Cryptosporidiosis in bovine and human health. *J Dairy Sci* 1998; **81**: 3036–3041.

[21] Lindsay DS, Upton SJ, Owens DS, Morgan UM, Mead JR, Blagburn BL. *Cryptosporidium andersoni* n. sp. (Apicomplexa: Cryptosporiidae) from cattle, Bos taurus. *J Eukaryot Microbiol* 2000; **47**: 91–95.

[22] Satoh M, Hikosaka K, Sasaki T, Suyama Y, Yanai T, Ohta M, et al. Characteristics of a novel type of bovine *Cryptosporidium andersoni*. *Appl Environ Microbiol* 2003; **69**: 691–692.

[23] Matsubayashi M, Kimata I, Abe N, Tani H, Sasai K. The detection of a novel type of *Cryptosporidium andersoni* oocyst in cattle in Japan. *Parasitol Res* 2004; **93**: 504–506.

[24] Kvác M, Ondráčková Z, Kvetonová D, Sak B, Vítovec J. Infectivity and pathogenicity of *Cryptosporidium andersoni* to a novel host, southern multimammate mouse (Mastomys coucha). *Vet Parasitol* 2007; **143**: 229–233.