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ORIGINAL ARTICLE

Detections of gastrointestinal parasites, including *Giardia intestinalis* and *Cryptosporidium* spp., in cattle of Banten province, Indonesia

Dyah Haryuningtyas Sawitri[1] · April Hari Wardhana[1,2,3] · Eny Martindah[1] · Fitrine Ekawasti[1,2] · Dias Aprita Dewi[1] · Bambang Ngaji Utomo[1] · Tomoyuki Shibahara[2,4] · Masahiro Kusumoto[3] · Masaharu Tokoro[5] · Kazumi Sasai[2,6] · Makoto Matsubayashi[2,3,6]

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Abstract Gastrointestinal parasites can induce low productivity in livestock by causing acute or chronic enteritis. Veterinarians make great efforts to design rational and effective hygienic protocols for both the prevention and treatment of diarrhea. Although prevalences can vary depending on the examined areas or the ages of the hosts, and the methods used for detections, it is helpful to accumulate data across many areas to evaluate parasitic distribution. A coprological survey in cattle was conducted in Tangerang, Banten Province of Indonesia, in order to determine the prevalence of the parasites, including those of diarrhea-associated diseases. Furthermore, the risk of transmission of *Giardia intestinalis* and *Cryptosporidium* spp. to human was genetically analyzed. Gastrointestinal parasites were detected in 87 of 109 cattle samples, including 85 carrying *Eimeria* spp., 36 carrying *Fasciola gigantica*, 35 carrying *Strongyloides* spp., 33 carrying *Paramphistomum* spp., and 15 carrying *Capillaria* spp. *Giardia intestinalis* and *Cryptosporidium* spp., parasites with zoonotic potential, were detected in 9 and 1 cattle samples, respectively. Molecular analyses identified the *G. intestinalis* isolate as a member of Assemblage E, which has been recently detected in humans in another country. These results may be helpful in understanding the hygienic risk affecting the livestock productivity and zoonotic potential of cattle in Indonesia.

Keywords Assemblage E · *Cryptosporidium* · *Giardia intestinalis* · Indonesia, Tangerang

Introduction

Gastrointestinal parasites, including Protozoa, Nematoda, and Trematoda, can negatively affect productivity in livestock (Corwin 1997; Grisi et al. 2014). These parasites cause acute or chronic enteritis and lead to watery or bloody (and sometimes lethal) diarrhea, especially in younger animals like calves and chicks. Thus, parasitic infections often result in economic losses due to reduced weight gain of the hosts or the need to administer therapeutic treatments (Cho and Yoon 2014). The infected hosts shed environmentally robust stages of the parasites in feces, including oocysts or cysts for the Protozoa and ova or encysted larvae for the Nematoda and Trematoda. These stages are resistant to commonly used disinfectants and can survive for several months or more, potentially being transmitted to other hosts that in turn serve as potential sources of further infection. Therefore, veterinarians and farmers make great efforts to determine the prevalence of such infections, and to design rational and effective

✉ April Hari Wardhana
wardhana24id@yahoo.com

✉ Makoto Matsubayashi
matsubayashi@vet.osakafu-u.ac.jp

[1] Indonesian Research Center for Veterinary Science, Bogor 16114, Indonesia

[2] Department of Veterinary Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka 598-8531, Japan

[3] Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Airlangga University, Surabaya 60115, Indonesia

[4] National Institute of Animal Health, NARO, Tsukuba, Ibaraki 305-0856, Japan

[5] Department of Parasitology, Graduate School of Medical Sciences, Kanazawa University, Kanazawa 920-8640, Japan

[6] Asian Health Science Research Institute, Osaka Prefecture University, Osaka 598-8531, Japan

hygienic protocols for both the prevention and treatment of diarrhea (Smith 2015).

To date, four surveys of gastrointestinal parasites have (to our knowledge) been conducted in cattle in some areas of Indonesia, including West Java, Central Java, and East Java (Estuningsih et al. 2009; Ananta et al. 2014; Ekawasti et al. 2019; Hastutiek et al. 2019). Regarding Protozoa, *Eimeria* spp. were the most frequently detected, and the prevalences were reported to range from 22.4 to 53.4%; in contrast, *Cryptosporidium* spp. were rarely found (0.5% and 0.6%) (Ananta et al. 2014; Ekawasti et al. 2019; Hastutiek et al. 2019). However, very limited information is available for other protozoan parasites, including *Giardia intestinalis*, and nematodes and trematodes in Indonesia. Because prevalences can vary depending on a number of parameters, such as the examined areas, the ages of the hosts, and the methods used for detections, it is helpful to accumulate data across many areas to evaluate parasitic distribution.

In Indonesia, saturated salt flotation methods like the Whitlock and McMaster method are commonly used due to their simplicity and rapidity. In the present study, we examined the protozoan parasites using the sugar flotation method, which is more sensitive (Ekawasti et al. 2019), and conducted immunofluorescence assays (IFAs) to confirm the presence of *Cryptosporidium* spp. and *G. intestinalis*. Here, intestinal parasites in cattle were investigated by the flotation method for Protozoa and Nematoda and by the sedimentation method for Trematoda, to understand the prevalence and hygiene status in cattle toward the improvement of production. Additionally, among these parasites, *Cryptosporidium* spp. and *G. intestinalis* are known to infect humans and a wide range of animals. To assess the potential zoonotic risk of transmission from animals to humans, molecular analyses such as PCR and sequencing are needed. Because genetic identification of detected parasites typically have not been performed previously in Indonesia, further molecular analysis of the isolates of *Giardia* and *Cryptosporidium* spp. was attempted to determine the species/genotype and potential risk to humans.

Materials and methods

Study area and samples

During February 2017, we collected 109 fecal samples from adult beef cattle in 11 villages of the Tangerang district of the Banten Province, located on the island of Java, Indonesia. The average annual temperature is 25 °C. No animals showed clinical symptoms when fecal samples were collected. All samples were taken from the rectums of cattle, placed in individual plastic bags, and stored at 4 °C until laboratory examination.

Fecal examinations

Fecal samples were examined by the sugar flotation centrifugation method, which was modified based on a previous report (Matsubayashi et al. 2005; Fujino et al. 2006), and by a sedimentation method (Charlier et al. 2008). For the flotation method, 1 g of fecal sample was diluted in 9 mL of distilled water and centrifuged at 800 × *g* for 5 min. The supernatant was discarded and 10 mL of sugar solution with a specific gravity of 1.2 [e.g., 100 g of sugar (Gulaku Indonesia, Lampung, Indonesia) was added into the 120 mL of distilled water] was added to the sediment, followed by centrifugation at 800 × *g* for 5 min. The floated material was transferred to a glass slide. We examined the entire smear by light microscopy under 200× or 400× magnification. For detection of trematode eggs, 3 grams of fecal samples were used for the sedimentation method, as reported previously (Charlier et al. 2008). 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 at least 10 min to allow the eggs to settle; and the supernatant then was discarded. This step was repeated twice. Finally, the collected sediments were stained with 5% methylene blue and observed by microscopy.

To confirm the presence of *Giardia* cysts or *Cryptosporidium* oocysts, the parasites were purified using the remaining feces (approximately 5–10 g). Briefly, feces were diluted in distilled water and filtered through a steel mesh. After centrifugation at 800 × *g* for 5 min, the above sugar solution was added to the sediments, and the mixture was overlaid with distilled water; the gradient then was centrifuged at 1200 × *g* for 10 min. The oocysts or cysts that floated on the surface of the sugar solution were recovered using a Pasteur pipette and were washed three times with distilled water. Finally, the purified oocysts were resuspended with 1–2 mL of phosphate-buffered saline and stored at 4 °C. The IFA was conducted using 10–20 µL of purified samples with a commercial *Cryptosporidium/Giardia* detection kit (EasyStain™; BTF, Australia) according to the manufacturer's instructions.

Molecular identification of *Giardia* and *Cryptosporidium* spp.

For DNA extraction, 400 µL of *Giardia* cysts or *Cryptosporidium* oocysts purified as described above were used. The cysts/oocysts were subjected to five cycles of freeze–thaw to release the genomic DNA, and the resulting suspensions were centrifuged at 5400 × *g* for 3 min. An

aliquot (200 μL) of each of the resulting supernatants then was processed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

For identification of *G. intestinalis*, diagnostic fragments were amplified using primer pairs G7 and G759, which are specific for the *Giardia β-giardin* gene (yielding a fragment of approximately 760 bp) (Cacciò et al. 2002) and GDH1 and GDH4, which are specific for the *glutamate dehydrogenase* gene (GDH) (yielding a fragment of approximately 770 bp) (Homan et al. 1998). For identification of *Cryptosporidium* spp., nested PCR with primer pairs F1 and R1 and F2 and R2 (targeting the *Cryptosporidium 18S ribosomal RNA* (18S rRNA) gene; yielding a fragment of approximately 770 bp) were performed as previously described (Xiao et al. 1999; Nagano et al. 2007). Additionally, PCR targeting for *Cryptosporidium oocyst wall protein* (COWP) genes (Spano et al. 1997) was conducted. All of the PCR-negative samples were amplified using Illustra GenomiPhi DNA Amplification Kit (GE Healthcare Life Sciences, Tokyo, Japan), and the PCRs were performed again using amplified DNAs.

PCR products were subjected to electrophoretic separation on an agarose gel, stained with ethidium bromide, and visualized on a UV transilluminator. PCR products from positive samples were sequenced in both directions using the amplifying primers and an ABI 3130 automated sequencer (Applied Biosystems Inc., CA, USA). The obtained sequences were aligned with the representative nucleotide sequences deposited in GenBank (https://www.ncbi.nlm.nih.gov/) using ClustalW with initial fixed parameter values (DNA Data Bank of Japan (DDBJ), http://clustalw.ddbj.njg.ac.jp/top-j.html). Homology searches using the obtained partial gene sequences were performed using the FASTA program (http://www.ddbj.nig.ac.jp/search/fasta-j.html) (Yui et al. 2014).

Results

Fecal samples from 30 farms in Tangerang, Banten province, Indonesia, were screened for the presence of gastrointestinal parasites. Among the 109 examined samples, 87 were positive (Table 1). The most prevalent parasites were *Eimeria* spp. (78.0%), followed by *Fasciola gigantica* (33.0%), *Strongyloides* spp. (32.1%), and *Paramphistomum* spp. (30.3%). Although the species of *Eimeria* could not be identified based on the morphological examinations, oval-shaped oocysts at a size of approximately 25 μm (resembling those of *E. bovis*) and spherical oocysts at a size of approximately 18 μm (resembling those of like *E. zuernii*) were detected. The cysts of *G. intestinalis* and oocysts of *Cryptosporidium* spp. (morphologically resembling *C. parvum*) were found in 9 and 1 samples, respectively, by IFA.

By PCR analyses, only a product specific for the *Giardia β-giardin* gene was obtained. Notably, this amplicon was obtained in only one (isolate No. 58) of the nine samples that had tested positive for *G. intestinalis* by IFA. The sequence of the isolates (Accession No. LC484286) possessed 2-bp or 4-bp differences from those of previous data [Assemblage E from pig in Czech Republic (Accession No. AY072729) or Assemblage E from sheep (Accession No. DQ116624)], respectively (Cacciò et al. 2002; Di Giovanni et al. 2006). The phylogenetic analyses revealed that the isolate was sorted with the cluster of Assemblage E (Fig. 1). The products of the other samples *G. intestinalis* samples and one *Cryptosporidium* spp. sample were not successfully amplified by PCR.

Discussion

Gastrointestinal parasites in cattle reared in the Tangerang district of Banten province, Indonesia, were investigated coprologically. *Eimeria* infections were highly common in the examined areas, representing the most abundant class of parasites detected. Although a molecular analysis of these *Eimeria* isolates could not be conducted, the oocysts were similar in size and morphology to those of *E. bovis* and *E. zuernii*, known pathogenic species. Recently, it has been reported that *E. bovis* is highly prevalent in other areas of Indonesia (Ekawasti et al. 2019). Therefore, the *Eimeria* spp. identified in the present study might represent potentially lethal agents capable of causing bloody diarrhea, especially in younger calves. The second-most-prevalent class of parasites, present in approximately 30% of the examined cattle, were *Strongyloides* spp., *Fasciola gigantica*, and/or *Paramphistomum* spp. None of these cattle exhibited clinical symptoms when the feces were collected. Thus, these animals may have been lightly infected with the parasites or may have possessed acquired immunity against the parasites. Furthermore, the parasites may not have been spreading to other animals because the farms investigated were relatively small and managed by the family units, harboring < 10 cattle each (data not shown).

Giardia intestinalis and *Cryptosporidium* were detected in 9 and 1 cattle, respectively. We were able to PCR amplify a corresponding organism-specific product from only one of the samples that were positive by IFA. This failure to obtain PCR products may indicate that that the number of the cysts and oocysts was small, that the genomic DNAs were recovered at concentrations too low to permit PCR, or that the extracted DNAs contain some PCR inhibitors. However, the successful PCR amplification did permit the first (to our knowledge)

Table 1 Prevalence of gastrointestinal parasite infection in cattle in the Tangerang district of the Banten province, Indonesia

Sub district	Names of villages	Nos. of farms	Nos. of cattle	Positive nos. of parasites	Protozoan			Nematoda			Trematoda	
					Eimeria spp.	Gi*[1]	Cp*[2]	*Strongyloides* spp.	*Capillaria* spp.	*Trichuris* spp.	*Fasciola gigantica*	*Paramphistomum* spp.
Panongan	Serdang Kulon	6	12	12	12	1	0	9	7	0	10	7
	Mekar Jaya	2	4	4	4	0	0	4	3	1	0	1
	Ranca Iyuh	1	7	7	7	0	0	4	0	1	2	2
	Ranca Kelapa	1	3	3	3	0	0	2	0	0	1	0
	Ciakar	1	3	3	3	1	0	0	0	0	3	3
Tigaraksa	Cileles	10	38	33	28	4	1	18	4	1	12	14
	Margasari	2	8	4	2	0	0	0	0	0	0	0
	Bantar Panjang	1	4	3	2	1	0	0	0	0	1	0
	Kadu Agung	1	3	0	0	0	0	0	0	0	0	0
Solear	Cikareo	4	18	16	14	2*[3]	0	9	1	0	7	4
Cisauk	Dandang	1	9	8	8	0	0	6	1	0	0	0
Total (%)		30	109	93 (85.3)	83 (76.1)	9 (8.3)	1 (0.9)	52 (47.7)	16 (14.7)	3 (2.8)	36 (33.0)	31 (28.4)

*[1]Gi; *Giardia intestinalis*, *[2]Cp; *Cryptosporidium* spp., *[3]One of samples was successfully identified as Assemblage E

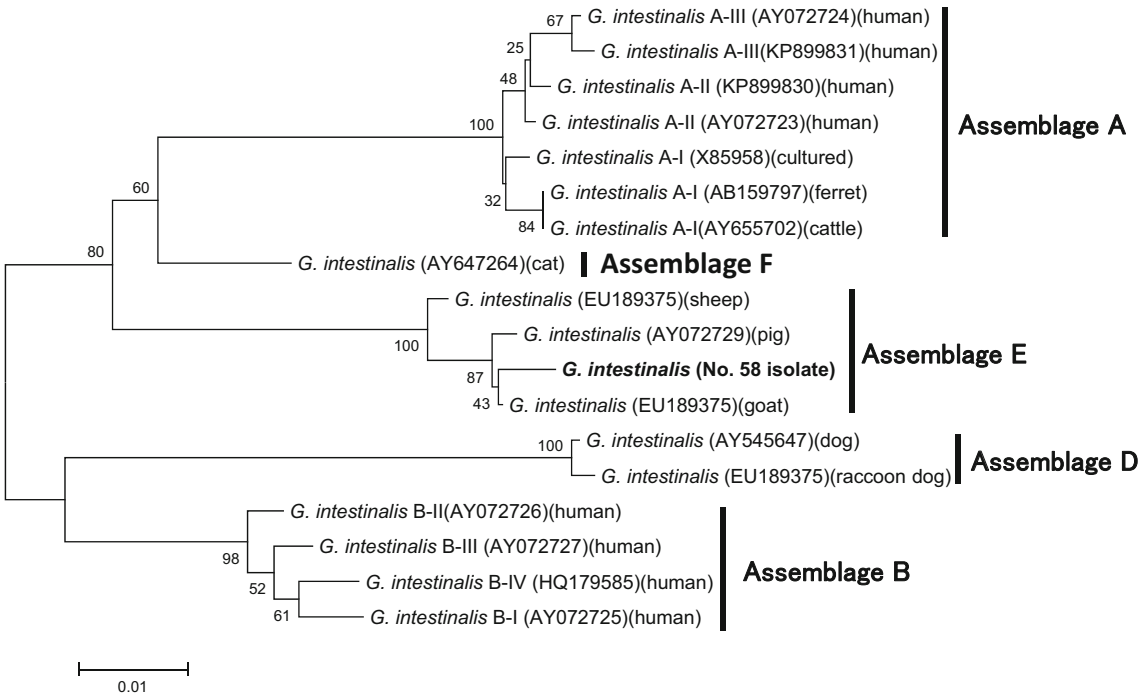

Fig. 1 Phylogenetic tree of *Giardia intestinalis* inferred by the neighbor-joining method using partial *Giardia β-giardin* gene sequences. Accession numbers and source hosts are shown in parentheses. The scale bar represents substitutions per nucleotide, and bootstrap values are indicated (N = 1000)

identification of an isolate from Assemblage E of *G. intestinalis* isolate from cattle in Indonesia. *G. intestinalis* is a species complex consisting of eight assemblages (A–H), with assemblages A and B the dominant assemblages in humans (Ryan and Zahedi, 2019). Notably, while Assemblage E is known to infect hoofed livestock, isolates of this assemblage recently have been shown to have zoonotic potential (Abdel-Moein and Saeed 2016). Although there have been some reports of *G. intestinalis* infections in humans and animals in Indonesia, only Assemblage B previously has been detected in this country, specifically in orangutans (Mynářová et al. 2016). Additionally, *Cryptosporidium* spp. has been detected in humans from several areas of Indonesia, including Jakarta (Katsumata et al. 2000; Kurniawan et al. 2009 and 2013), which is close to the Tangerang district examined in the present study. Therefore, further genetic epidemiological surveys across wider geographic regions are needed to assess the zoonotic risk of these parasites.

Conclusion

To our knowledge, only four papers have reported surveys of the parasites in cattle in Indonesia, specifically in West Java, Central Java, East Java, and Yogyakarta as described in Introduction. Although we cannot easily compare the prevalences among these various reports, all of these results together are expected to facilitate our understanding of the potential risks affecting livestock production and human health in Indonesia.

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Author's contribution SDH, WAH, ME, EF, DDA, and MM collected the fecal samples, examined the parasites, and summarized the results and information of the farm. WAH and UBN organized the survey at the examined areas. ST, KM, SK, and MM performed molecular analyses of the parasites. SDH, TM, and MM mainly provided the manuscript. SDH, TM, WAH and MM equally contributed in the manuscript writing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The research work was conducted under the Indonesian Research Center for Veterinary Science, Bogor, Indonesia. The handling of animals in the study was approved by the ethics committee of Indonesia Agency for Agricultural Research and Development, Ministry of Agriculture, Indonesia (No. Balitbangtan/BB Litvet/AT_HL/01.01/2017).

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