

Identification of *Cryptosporidium* from Dairy Cattle in Pahang, Malaysia

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Abstract: *Cryptosporidium*, a protozoan parasite, can cause cryptosporidiosis which is a gastrointestinal disease that can infect humans and livestock. Cattle are the most common livestock that can be infected with this protozoan. This study was carried out to determine the prevalence of *Cryptosporidium* infection in cattle in Kuantan, Pahang, Malaysia and to find out the association between the occurrence of infection and 3 different ages of cattle (calves less than 1 year, yearling, and adult cattle). The samples were processed by using formol-ether concentration technique and stained by modified Ziehl Neelsen. The results showed that 15.9% (24/151) of cattle were positive for *Cryptosporidium* oocysts. The occurrence of *Cryptosporidium* in calves less than 1 year was the highest with the percentage of 20.0% (11/55) followed by yearling and adult cattle, with the percentage occurrence of 15.6% (7/45) and 11.8% (6/51), respectively. There was no significant association between the occurrence and age of cattle and presence of diarrhea. Good management practices and proper hygiene management must be taken in order to reduce the infection. It is highly important to control the infection since infected cattle may serve as potential reservoirs of the infection to other animals and humans, especially animal handlers.

Key words: *Cryptosporidium*, cattle, age, Pahang, Malaysia

Cryptosporidium sp. is a protozoan parasite that can cause gastrointestinal infection known as cryptosporidiosis. This parasite is responsible for veterinary problems particularly enteric illness outbreaks in livestock animals [1]. In farm animals, cattle are recognized as the most common mammalian species that can be infected by *Cryptosporidium* [2-4]. It is known since 1980s that calves and cattle have been identified as an important reservoir for zoonotic *Cryptosporidium* spp. [5]. The major *Cryptosporidium* sp. that infects calves is *Cryptosporidium parvum* which can cause severe watery, yellowish, and foul-smelling acute diarrhea that may lead to morbidity and mortality [6].

In Malaysia, little is known about *Cryptosporidium* spp. infection in cattle especially in Kuantan, Pahang. The first report of

the incidence of *C. parvum* among calves in Malaysia was reported by Lee et al. [7] who found 4 out of 11 diarrheic calves in Johor had the infection. For more than a decade, there was no study done on *Cryptosporidium* in cattle in Malaysia until 2005. Farizawati et al. [8] reported that *Cryptosporidium* oocysts from cattle had contaminated rivers in Sungai Langat Basin, and Halim et al. [9] reported the first data on *Cryptosporidium* deer-like genotype from cattle in Selangor.

Since there are few studies on *Cryptosporidium* infection in cattle in Kuantan area, the present study attempted to identify the occurrence of *Cryptosporidium* in cattle and to find out the association of the occurrence with age groups of cattle and the presence of diarrhea.

This cross-sectional study was conducted at a dairy cattle farm (3°43'01.7"N 103°00'41.6"E) located in Ulu Lepar, Kuantan Pahang, Malaysia (Fig. 1). Kuantan is located at east coast of Peninsular Malaysia, and the climate is tropical rainforest climate. The average maximum temperature is 31°C, and the average minimum temperature is 23°C [10].

Ethical clearance was obtained from the Institutional Ani-

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Fig. 1. The sampling site in Kuantan, Pahang, Malaysia.

mal Care and Use Committee (IACUC), International Islamic University of Malaysia (IIUM). A total of 151 fecal samples were collected between May 2015 and August 2015. The fecal samples were collected according to the age group: 55 samples from calves (less than 12 months), 45 samples from yearlings (12-24 months), and 51 samples from adults (more than 24 months). The fecal samples were collected directly from the rectum of the animals by using sterile plastic gloves. The samples were transferred into stool containers and preserved in 10% formalin. For each animal, the sampling date, age, identification tag, gender, and consistency of feces (score 0-1, 0: absence of diarrhea, 1: presence of diarrhea) were recorded. The samples were immediately transported to the laboratory and stored at 4°C before analysis.

The fecal samples were processed by using formol-ether concentration technique based on the method of Allen and Ridley [11] with slight modifications. Approximately 1 g of stool sample was emulsified in 4 ml of 10% formal saline by using an applicator stick. The suspension was filtered through a sieve or gauze into a beaker. A 3-4 ml formal saline was added followed by an addition of 2.5 ml diethyl ether to the formalized solution. The samples were centrifuged at 500 g for 30 sec and continued with 500 g for another 20 sec. Finally, the supernatant was removed, and 1 or 2 drops of the resuspended pellet were smeared onto a microscope slide and stained with modified Ziehl Neelsen for *Cryptosporidium* examination. Later, the slides were air-dried and viewed under the x 1,000 objective lens of a light microscope.

Statistical analysis were performed by using Statistical Package for Social Sciences for windows SPSS (version 16). Chi-square test was used to compare the occurrence of infection between the age group of cattle, and Fisher's exact test was used to find the association between the occurrence of infec-

Table 1. The occurrence of *Cryptosporidium* in cattle in different age groups of cattle

Age	Total sample	Positive	Occurrence (%)	<i>P</i> -value
Calves	55	11	20	
Yearling	45	7	15.6	0.51
Adult	51	6	11.8	

If *P*-value of less than 0.05 was considered statistically significant.

Table 2. The association between the presence of diarrhea and the occurrence of infection

Age	Total sample	Positive	Occurrence (%)	<i>P</i> -value
Diarrheic	23	4	17.4	0.764
Non-diarrheic	128	20	15.6	

P-value of less than 0.05 was considered statistically significant.

tion and the presence of diarrhea. A *P*-value of <0.05 was considered as the level of significance.

Referring to Table 1, the results showed that the prevalence of *Cryptosporidium* infection in calves less than 1 year was the highest compared to those in yearling and adult cattle. However, there was no significance difference ($P > 0.05$) between the age group and occurrence of *Cryptosporidium*. Analysis indicated that the occurrence of *Cryptosporidium* in calves less than 1 year was the highest with the percentage of 20.0% while in yearling and adult cattle, the percentage of occurrence was 15.6 % and 11.8%, respectively.

Of the total 151 samples collected, 23 were diarrheic samples, and 128 were non-diarrheic ones. As shown in Table 2, from 23 diarrheic samples, 4 (17.4%) were positive for infection while out of 128 non-diarrheic samples, 20 (15.6%) were positive for *Cryptosporidium* oocysts. However, the results showed that there is no significant difference between the presence of diarrhea and occurrence of infection.

Microscopic analysis of 151 fecal samples showed that 24 cattle (15.9%) were positive for *Cryptosporidium* infection. Studies on *Cryptosporidium* infection in cattle population have been conducted from other researchers. The results were almost similar with former studies in Egypt, Poland, and China with the overall prevalence of 13.6%, 17.0%, and 16.0%, respectively [12-14]. So far, there were only 2 data collected from Pahang area which was done in Kuantan and Jerantut, Pahang. Yap et al. [15] reported a lower prevalence of *Cryptosporidium* infection in Kuantan and Jerantut areas, Pahang as compared to the present study which was 2.7% and 2.9%, respectively. Another study conducted in Johor by Muhid et al. [16] reported that the overall prevalence of *Cryptosporidium* infection in

pre-weaned and post-weaned calves was 27.1% (65/240) indicating a higher prevalence of infection compared to the present study. The dissimilarity in the prevalence among countries may be due to a different amount of stocking rate and different management system in the farm [17].

In summary, calves less than 1 year recorded the highest occurrence of *Cryptosporidium* sp. followed by yearlings and adult cattle. Similar high prevalences of *Cryptosporidium* in young calves compared to adult cattle have been reported by other studies [18-20]. This finding is most likely due to the low immune system function of young neonates as suggested by Maurya et al. [21]. Young animals have become more vulnerable to infection and disease, while infections in adults usually show no symptoms [22]. This result is in agreement with other studies which found no statistically significant association between infection and diarrhea [23-26]. On the other hand, other previous studies have reported that *Cryptosporidium* sp. infection is associated with the occurrence of diarrhea [27].

The present study has succeeded to achieve the objective of the research. This research has indicated that there is *Cryptosporidium* infection in cattle in Kuantan, Pahang. The findings suggested that control and prevention measures must be taken in order to reduce the infection among cattle. It is highly important to control the infection since cattle infected with parasites may serve as potential reservoirs of the infection to other animals and humans especially animal handlers. Good management practices and proper hygiene managements are the most effective weapons to combat *Cryptosporidium* spp. infections.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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