Prevalence of Giardia spp. infection in pre-weaned and weaned calves in relation to management factors

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Abstract

Two hundred and forty calf faecal samples from 16 Malaysian farms were screened by PCR for *Giardia* spp. The overall prevalence was 12.5% and the overall farm prevalence was 68.8% (11/16 farms). The prevalence in pre-weaned and weaned calves was 16.7% and 8.3%, respectively. Sequence analysis of 25 isolates identified all as *G. duodenalis* assemblage E. Management factors associated with an increased risk of infection with *Giardia* spp. included keeping weaned calves in pens with sand floors and calf age. Keeping pre-weaned calves in pens with concrete floors and calving in single cow calving areas decreased the risk.

Keywords: *Giardia*; Prevalence; Calves; Genotyping; Management factors

Giardia duodenalis is a common protozoal parasite in humans and veterinary species and comprises eight major genetic assemblages, with A and B infecting humans (Caccio and Ryan, 2008 and Lasek-Nesselquist et al., 2010). Little is known about *Giardia* spp. in cattle or the role of cattle in the transmission of infection to humans. The aim of this study was to determine the prevalence, assemblages and management factors related to *Giardia* spp. infections in pre-weaned and weaned dairy calves in Malaysia.

Sixteen dairy farms in the Kluang and Johor Bahru districts were included in the study. Eight of these were intensive farms on which animals were kept indoors and eight were semi-intensive farms on which animals were permitted to graze outside during the day. A total of 240 faecal samples were collected from the rectum of pre-weaned ($\leqslant$4.5 months of age) and weaned (4.5–12 months of age) calves from October 2008 to February 2009. Data on demographic and management factors were obtained from farmers using a questionnaire. Permission for the study was granted by Murdoch University Animal Ethics Committee (R2193/08) and Human Ethics Committee (2008/205).

Genomic DNA was extracted from 200 mg of each faecal sample using the QIAamp DNA Mini Stool Kit (Qiagen). Nested PCR assays were used for amplification of 18S rRNA and*gdh* (Read et al., 2002 and Read et al., 2004). Purified PCR products were sequenced using the Big Dye Cycle Sequencing Kit (Applied Biosystems) and nucleotide sequences were aligned using Clustal W (Thompson et al., 1997). Statistical analyses were performed using SPSS version 17.0.

A total of 30/240 bovine faecal samples were positive by 18S and *gdh* PCR for *Giardia* spp. giving an overall prevalence of 12.5% (Table 1). The prevalence was higher in pre-weaned calves (16.8%) than weaned calves (8.3%) (χ^2 = 4; df = 1,1; P = 0.04). Eleven out of 16 farms were positive for *Giardia* spp, giving a farm prevalence of 68.8%; the prevalence between farms ranged from 0% to 37.5%.

There were no significant differences between the prevalence of *Giardia* spp. on intensive farms (10.8%, range 0–30.4%) compared to semi-intensive farms (14.2%, range 0–37.5%) (χ^2 = 2.55; df = 1,1; P = 0.11; Table 1), in pre-weaned calves on intensive farms (10%) compared to semi-

intensive farms (21.4%) (χ^2 = 2.60; df = 1,1; P = 0.11) or in weaned calves on intensive farms (11.4%) compared to semi-intensive farms (4%) (χ^2 = 2.04; df = 1,1; P = 0.15). *Giardia* spp. were detected in calves aged 1–9 months old, with the highest prevalence in 2-month-old calves (29.2%; 95% CI 11–47.4%) (Fig. 1); there were significant differences between age groups (χ^2 = 16.17; df = 1,8; P = 0.034).

Univariate logistic regression analysis of 79 management factors revealed that 13 were significantly associated with *Giardia* spp. infection in dairy calves ($P < 0.05$). These were: pre-weaned calves, calves separated according to gender, calves born in single and multi-cow calving areas, calves not separated from their dam within 12 h of birth, pre-weaned calves kept on concrete floors, calves receiving starter feed in the first week of life, weaned calves kept in multi-age groups, weaned calves kept in pens with concrete floors or on sand floors, vehicle disinfection at the farm entrance and abnormal faecal consistency or colour. The distance of a farm from other cattle farms and the age of calves were also significantly associated with *Giardia* spp. infection ($P < 0.05$).

Multivariable logistic regression analysis identified that using a pre-weaning pen with a concrete floor, using a weaning pen with a sand floor, a single cow calving area, calves <5 months of age and administering anthelmintics frequently (at least four times a year) significantly increased the risk of infection with *Giardia* spp. ($P < 0.05$) (Table 2).

The total prevalence of *Giardia* spp. in dairy calves in Malaysia was 12.5%, which is lower than in studies in other countries, in which prevalences ranged from 14% to 100% (O'Handley and Olson, 2006, Caccio and Ryan, 2008 and Santín et al., 2009). However, in those studies, faecal samples were only collected from calves <6 months old and from diarrhoeic calves. The herd prevalence (at least one positive animal detected on a farm) in our study was 68.8%, similar to herd prevalences reported in previous studies (53–100%) (O'Handley and Olson, 2006). In our work, the prevalence of *Giardia* spp. was higher in pre-weaned calves (16.8%) than in weaned calves (8.3%), similar to a recent study in the USA (Santín et al., 2009).

Giardia sequences from 18S rDNA (*n* = 10), *gdh* (*n* = 9) or both (*n* = 6) were obtained from 25/30 positive samples from pre-weaned calves (*n* = 16) or weaned calves (*n* = 9); all were assemblage E. Surveys of dairy cattle worldwide have reported predominately assemblage E, with lower frequencies of assemblage A and occasionally assemblage B (O'Handley and Olson, 2006, Caccio and Ryan, 2008 and Santín et al., 2009). Assemblage E has not been identified in humans and thus its zoonotic risk appears to be minimal (Caccio and Ryan, 2008).

Pre-weaned calves kept in pens with a concrete floor had a lower prevalence of infection with *Giardia* spp. This is likely to be related to the type and frequency of cleaning, since cement floors were washed thoroughly with water daily using a pressure hose, whereas other types of floors, such as sand, were swept only occasionally.

Calves born in a single cow calving area were less likely to be infected with *Giardia* spp. compared to those born in a multiple cow calving area, presumably due to increased potential for transmission. There was a higher risk of infection in calves that were more frequently administered benzimidazole and fenbendazole, contrasting with previous studies reporting that these anthelmintics were effective against giardiasis (O'Handley and Olson, 2006 and Geurden et al., 2010). High levels of contamination with *Giardia*spp. cysts may result in frequent re-infection, leading to failure of anthelmintic treatment (O'Handley et al., 2003).

This is the first study to determine the prevalence of *Giardia* infection in dairy cattle in Malaysia and the relationship of management factors to infection. The prevalence of*Giardia* in dairy calves was high (especially in pre-weaned calves) and all PCR positive samples were identified as the non-zoonotic assemblage E. Further studies should be conducted on larger sample sizes from different locations, with additional data on temperature, rainfall and soil type, herd size and other management practices, to obtain more accurate data on factors associated with *Giardia* spp. infection in cattle.

Conflict of interest statement

None of the authors of this paper has any financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of this paper.

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Table 1. Prevalence of *Giardia* spp. in pre-weaned and weaned calves in Malaysia according to farming system.

Farming system	**Pre-weaned calves**		**Post-weaned calves**		Total (95% confidence interval), %
	Number of positive calves/number of calves tested	Prevalence (95% confidence interval), %	Number of positive calves/number of calves tested	Prevalence (95% confidence interval), %	
Intensive	5/50	10 (1.7–18.3)	8/70	11.4 (4.0–18.9)	10.8 (5.3–16.4)
Semi-intensive	15/70	21.4 (11.8–31.0)	2/50	4 (0–9.4)	14.2 (7.9–20.4)
Total	20/120	16.7 (10.0–23.3)	10/120	8.3 (3.4–13.3)	12.5 (8.3–16.7)

Table 2. Binary logistic regression for management risk factors associated with infection with *Giardia* spp. in dairy calves in Malaysia.

Variables	ß estimates	Odds ratios (95% confidence intervals)	*P* value
Pre-weaning pen with a concrete floor	−1.89	0.15 (0.05–0.47)a	0.001
Post-weaning pen with a sand floor	2.69	14.67 (2.45–87.80)b	0.003
Single cow calving area	−1.86	0.16 (0.04–0.63)a	0.009
Calves <5months old	1.5	4.47 (1.58–12.65)b	0.005
Anthelmintics given to the calves at least four times/year	2.08	7.96 (2.22–28.52)b	0.001
Constant	−3.01	0.05	0

Fig. 1. Prevalence of *Giardia* spp. in Malaysian calves according to age of calf.