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Prevalence of and management factors contributing to *Cryptosporidium sp.* infection in pre-weaned and post-weaned calves in Johor, Malaysia.

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Abstract

A cross-sectional study was carried out to identify species and determine the prevalence of *Cryptosporidium sp*. shedding in pre-weaned and post-weaned dairy calves and to identify management factors that may be contributing to disease. A total of 240 calf faecal samples were collected from 16 farms in two districts in Johor, Malaysia and screened by PCR. The overall *Cryptosporidium* prevalence was 27.1%. The prevalence of *Cryptosporidium* species in pre-weaned calves was 32.4% for *C. parvum,* 26.5% for *C. bovis*, followed by *C. andersoni* (20.6%), *C. ryanae* (11.8%) and mixed sp. (8.8%). The prevalence of *Cryptosporidium* species in post-weaned calves was 35% for *C. bovis* followed by *C. andersoni* and *C. ryanae* (30% each) and mixed sp. (5%). Subtyping analysis of eight of the 11 *C. parvum* isolates at the *gp60* locus identified five isolates as IIdA15G1, one as IIa18A3R1 and two isolates as IIa17G2R1. Management factors that increased the risk of *Cryptosporidium* infection included having other cattle farms close by, feeding calves with saleable milk, keeping pre-weaned calves in pens with slatted floors and keeping post-weaned calves in pens with a sand floor.

Keywords: Cryptosporidium; Prevalence; Calves; Genotyping; Zoonoses; Management factors.

1. Introduction

During the past decade, *Cryptosporidium* has emerged as an important pathogenic enteric protozoan parasite in Malaysia with high rates of cryptosporidiosis reported in humans, especially in children and AIDS patients (Menon et al., 2001; Lim et al., 2008).

Currently 23 different species of *Cryptosporidium* and over 40 genotypes are recognized, with new genotypes continually being identified (Xiao, 2010; Fayer et al., 2010). Of these, *C. hominis* and *C. parvum* are responsible for most human infections (Xiao, 2010). Over the past 20 years, cattle were thought to be one of the main reservoir hosts for the zoonotic *C. parvum*, however, recent molecular studies have indicated that cattle are infected with at least five *Cryptosporidium* parasites; *C. parvum, C. bovis, C. andersoni* and *C. ryanae* (previously *Cryptosporidium* deer like genotype) and *C. suis* (Xiao and Fayer, 2008).

Few studies have combined molecular characterisation with risk factor analysis in cattle (Hamnes et al., 2006; Nguyen et al., 2007; Paul et al., 2008; Winkworth et al., 2008). In Malaysia, little is known about *Cryptosporidium* sp. in cattle. A recent study on a small number of isolates (n = 50) has shown that *Cryptosporidium* is prevalent (36%) in cattle in Malaysia and that *C. parvum* is the most common species (Halim et al., 2008). However, no prevalence studies have been conducted in different localities and between farms with different management factors. The aim of the present study was to determine the prevalence, genotypes and management factors related to *Cryptosporidium* infections in pre-weaned and post-weaned dairy cattle in Malaysia.

2. Materials and Methods

2.1 Sources of isolates and collection of specimens

Farms were selected based on the total cattle population and the farming system; i.e. intensive or semi-intensive. Intensive farms were defined as farms where animals were kept inside the barn 24 hours a day, without grazing and feed supplements were given. Semi intensive farms were defined as farms where animals were permitted to graze outside the barn during the day and were kept in the barn during night-time. Seven farms from the Kluang district and nine farms from the Johor Bahru district were included in the study. Semi-intensive farms were chosen largely on the basis of population size to ensure that an adequate number of samples could be collected. However, as only a small number of intensive farms operate in these regions, all intensive farms were selected.

A total of 120 faecal samples were collected directly from the rectum from calves aged one day to 12 months old from each of the two districts between October 2008 and February 2009 (total 240). An equal number of faecal samples were collected from pre-weaned calves (≤ 4.5 months of age) and from post-weaned calves (> 4.5 months but ≤ 12 months of age). Permission to collect samples from the animals was obtained from the Murdoch University Animal Ethics Committee (R2193/08).

2.2 Survey of farming practices

A questionnaire was designed and administered to farmers to collect data on demographic, management and health factors hypothesized to be associated with the risk of infection with *Cryptosporidium* sp. in dairy herds. Permission to undertake the questionnaire was obtained from the Murdoch University Human Ethics Committee (2008/205).

2.3 DNA isolation and PCR screening

Genomic DNA was extracted from 200mg of each faecal sample using a QIAamp DNA Mini Stool Kit (Qiagen, Hilden, Germany). Eluted DNA was stored at -20 °C until required. All samples were screened using a two-step nested PCR at the 18S rRNA as previously described (Ryan et al., 2003). Sub-genotyping of *C. parvum* isolates was performed using a two-step nested PCR to amplify a ~832 bp fragment of the *gp60* gene as described (Strong et al., 2000; Peng et al., 2003). Purified PCR products were sequenced using an ABI Prism^{TM} Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California) according to the manufacturer's instructions. Nucleotide sequences were analyzed using Chromas lite version 2.0 (http://www.technelysium.com.au) and aligned with reference sequences from GenBank using Clustal W (http://clustalw.genome.jp/).

2.4 Statistical analysis

Prevalence and their 95% confidence intervals were calculated using the normal approximation method (Thrusfield, 2005). Statistical analyses were performed using SPSS version 17.0 (SPSS inc. Chicago, USA) to determine if there were any associations between the presence of *Cryptosporidium* sp. and the factors surveyed in the questionnaire. Univariable analyses conducted included chi-square tests for independence, Fisher's exact tests and ANOVA. Univariable factors with a $P \leq 0.20$ were offered to a multivariable logistic regression model. Backward elimination was used to determine which factors could be dropped from the multivariable model. The likelihood-ratio test statistic was calculated to determine the significance at each step of the model building. Because of the likely presence of additional variation due to the clustering of animals into herds, herd was incorporated as a random effect in the model. The goodness-of-fit for the random effects was evaluated by comparing the deviance and the change in the degrees of freedom. The level of significance for a factor to remain in the final model was set at 5%.

3. Results

3.1 Cryptosporidium prevalence in intensive and semi-intensive farms

A total of 240 cattle faecal samples were screened using PCR amplification at the 18S rDNA gene locus for the presence of *Cryptosporidium*. Sixty-five samples were positive for *Cryptosporidium* giving a total prevalence of 27.1% (95% CI: 21.5, 32.7%). The pre-weaned calf prevalence was 30.8% (95% CI: 22.6, 39.1%) and was similar to the post-weaned calf prevalence at 23.3% (95% CI: 15.8, 30.9) ($\chi2$=1.92, df=1,1, P=0.17) (Table 1). Twelve farms were positive for *Cryptosporidium* out of the 16 farms selected, with an overall farm prevalence of 75% (95% CI: 53.8, 96.2%). Between farms, the prevalence ranged from 0% to 64.5%. Intensive farms had a higher prevalence of *Cryptosporidium*; 31.7% (95% CI: 23.3, 40%) compared with semi intensive farms, which had an overall prevalence of 22.5% (95% CI: 15, 30%) but this difference was not significantly different ($\chi2$=2.55, df=1,1, P=0.11). The prevalence of *Cryptosporidium* in intensive farms ranged from 0% to 64.5%. The highest prevalence of *Cryptosporidium* in pre-weaned and post-weaned calves in intensive farms was 62.5%, (95% CI: 38.8, 86.2%) and 66.7%, (95% CI: 42.8, 90.5%) respectively. The prevalence of *Cryptosporidium* in semi-intensive farms ranged from 0% to 43.8%. The highest prevalence of *Cryptosporidium* in pre-weaned and post-weaned calves in semi-intensive farms was 42.9% (95% CI: 16.9, 68.8%) and 60%, 95% (CI: 17.1, 100%) respectively.

Fifty-four partial 18S rRNA sequences were obtained from the sixty-five positives. Of these, 16 (29.6%) were *C. bovis,* 13 (24.1%) were *C. andersoni*, 11 (20.4%) were *C. parvum*, 10 (18.5%) were *C. ryanae*, and 4 (7.4%) were mixed species (Table 2).

3.2 Prevalence of Cryptosporidium species in pre-weaned and post-weaned calves

Of the 37 positives from the 120 pre-weaned calf faecal samples, thirty-four sequences were obtained; 32.4% (11/34) were *C. parvum,* 26.5% (9/34) were *C. bovis*, 20.6% (7/34) were *C. andersoni*; 11.8% (4/34) were *C. ryanae* and 8.8% (3/34) were mixed species (*C. parvum* and *C. ryanae* in two calves aged 2 months and *C. parvum* and *C. bovis* in one 3 month old calf). Twenty sequences were obtained from the 28 positives from post-weaned calves screened; 35% (7/20) were *C. bovis,* 30% (6/20) were *C. andersoni* and *C. ryanae* respectively and 5% (1/20) was a mixed infection (*C. andersoni* and *C. bovis)* (Table 2). *Cryptosporidium parvum* was not detected in the post-weaned calves. The highest prevalence of *Cryptosporidium* sp. (45.8%), was detected in calves less than one-month old. The second highest prevalence (38.1%) was detected in four-month old calves. However the proportion of *Cryptosporidium* positive calves in the different age groups was not significantly different ($\chi 2$=7.89, df=1,9, P=0.54).

Cryptosporidium parvum was only isolated from calves aged <1 to 4 months old, with the highest peak at 33.3% detected in calves aged less than one-month old. *Cryptosporidium bovis* and *ryanae* were isolated from calves <1 month to 10 months old. *Cryptosporidium andersoni* was only detected in calves three-months and older with the highest peak, 15.4% in 10 to 12 month old calves. In calves ≥ 10 months, only *C. andersoni* was detected. ($\chi 2$=10.55, df=1,9, P=0.31).

3.3 Subtyping of C. parvum isolates at the gp60 locus.

At the *gp60* locus, eight of 11 *C. parvum* isolates were successfully sequenced. Of these, five isolates were IIdA15G1, one was IIa18A3R1 and two isolates were IIa17G2R1.

3.4 Risk factor analysis

Univariate analysis revealed that 10 management factors were significantly associated with the prevalence of *Cryptosporidium* infection in dairy calves (P<0.05). The management factors were; farms that didn't treat waste effluent/faeces, pre-weaned calves kept in groups, pre-weaned calves kept in pens with slatted floors, calves that received colostrum from their mother, calves that received starter feed in the first week of life, post-weaned calves that were kept in pens with concrete floors and on sand floors, faecal appearance not normal, faecal appearance fluid and watery and lastly faecal consistency foamy, mucous and sticky. Four continuous variables related to management were significantly associated with *Cryptosporidium* infection in dairy calves (P<0.05) (distance of farm from the nearest cattle farm, distance from other livestock farms, number of treatments given to calves per year and number of doses of anthelmintics given to calves per year).

Multivariable analysis identified that other cattle farms nearby, feeding pre-weaned calves with saleable milk, using slatted floors and sand floors significantly increased the risk of *Cryptosporidium* infection (Table 3). Treating with antibiotics at least 3 times/year, administering anthelmintics at least 4 times/year and washing feeding utensils were associated with a lower probability of *Cryptosporidium* infection.

4. Discussion

In the present study, the herd prevalence for *Cryptosporidium* (at least one positive animal detected in a farm) was 75%. This is in agreement with recent cross-sectional studies, which reported herd prevalences ranging between 53 and 100% (Santin et al., 2004; Geurden et al., 2006; Hamnes et al., 2006; Maddox-Hyttel et al., 2006; Brook et al., 2008; Trotz et al., 2008; Larsen et al., 2009; Silverlas et al., 2010). In the present study there was no association between prevalence of infection and farm type with prevalences of 28% and 26.4%, respectively in intensive and semi intensive systems. This is in agreement with another study in central Spain which reported prevalences of 45.9% and 50.4% respectively in intensive and semi intensive management systems (Castro-Hermida et al., 2002).

There was also no association between prevalence of infection in pre-weaned and post-weaned calves with prevalences of 30.8% and 23.3%, respectively. This finding is in contrast with a number of studies that reported a significant association between age and *Cryptosporidium* prevalence with the highest prevalence reported in pre-weaned calves (Santin et al., 2004; Geurden et al., 2006; Trotz et al., 2007; Brook et al., 2008; Paul et al., 2008; Santin et al., 2008). The prevalence in pre-weaned calves in the present study correlates well with previous cross-sectional studies with prevalences ranging from 20 to 59% (Santin et al., 2004; Geurden et al., 2006; Trotz et al., 2007; Brook et al., 2008; Paul et al., 2008; Santin et al., 2008; Trotz et al., 2008; Khan et al., 2010), although the age of calves sampled and the diagnostic tests used varied between studies.

In the present study the most prevalent species in pre-weaned calves was *C. parvum* (32.4%) and *C. bovis* (26.5%), followed by *C. andersoni* (20.6%) and *C. ryanae* (11.8%). *Cryptosporidium parvum* was only isolated in pre-weaned calves with the highest prevalence in calves less than one month old (33.3%). In some studies, it has been reported that the zoonotic *C. parvum* is responsible for the majority of *Cryptosporidium* infections in pre-weaned calves and only a small percentage of *Cryptosporidium* infections in post-weaned calves and heifers (which were mostly infected with *C. bovis, C. andersoni* and *C. ryanae)* (Santin et al., 2004; Thompson et al., 2007; Geurden et al., 2008; Halim et al., 2008; Santin et al., 2008). Other studies however have reported that *C. bovis* was the most common species found in pre-weaned calves (Feng et al., 2007; Feltus et al., 2008; Silverlås et al., 2010). A number of studies have reported no significant correlation between the calf age, oocyst excretion and the species/genotypes of *Cryptosporidium* detected (Geurden et al., 2006; Langkjaer et al., 2007; Winkworth et al., 2008).

In the present study, subtyping of 8 *C. parvum* isolates at the *gp60* locus identified IIdA15G1 (n=5), IIaA18G3R1 (n=1) and IIa17G2R1 (n=2). Recent subtyping studies shows that the *C. parvum* subtype families IIa and IId have been found in both humans and animals (cf. Xiao, 2010). Subtypes IIa A17G2R1 and

A18G3R1 are common in humans and animals in Australia, Canada and the United Kingdom, however, the IId subtype is less commonly reported in animals and has only been reported in cattle in Hungary, Portugal and Egypt and lambs and goats in Spain (Xiao, 2010, Amer et. al., 2010). Subtyping of *Cryptosporidium* positives from farm workers from the same farms is necessary to better understand the role that calves play in the zoonotic transmission of cryptosporidiosis in Malaysia.

In the present study, calves from farms that were adjacent to each other (less than 2.5km) were 8 times more likely to be infected with *Cryptosporidium* than calves from more isolated farms, presumably due to contamination of grazing land or water sources. Post-weaned calves kept on a sand floor were nearly five times more likely to be infected with *Cryptosporidium* when compared with post-weaned calves kept on a cement floor. This is most likely directly related to the type and frequency of cleaning, i.e. cement floors were washed thoroughly compared with other types of flooring (sand, earth or gravel). In addition, the viability of oocysts in soil is approximately two months, increasing the likelihood of calves ingesting infective oocysts (Lim et al., 1999). Surprisingly, pre-weaned calves reared on slatted floors were nearly seven times more likely to be infected with *Cryptosporidium*. In addition, calves from farms that frequently treated their animals for gastrointestinal problems with antibiotics (at least 3 times/year) and administered anthelmintics to calves more frequently (at least 4 times/year) were four times less likely to be infected with *Cryptosporidium.* However, some of the associations might be due to underlying confounding factors as the number of farms examined was limited and therefore the study lacks sensitivity.

Pre-weaned calves fed with saleable milk also had a higher probability of becoming infected with C*ryptosporidium*. This was also reported in another study that indicated that feeding milk (other than fresh colostrum) to calves increased the risk of infection (Mohammed et al., 1999). It is possible that the milk may become contaminated due to contaminated feeding bottles or contamination of milk during the milking process. There have been a number of cryptosporidiosis outbreaks linked with the consumption of raw milk and fermented dairy products made from raw milk (Xiao and Fayer, 2008).

The present study also indicated that farms that washed feeding utensils with disinfectant had a lower prevalence of infection with *Cryptosporidium*. This finding was in agreement with other studies that have reported that calves in pens, which were not disinfected were at a higher risk of infection compared with calves from pens, which were disinfected (Castro-Hermida et al., 2002; Hamnes et al., 2006). Again, it may be related to better overall management on the farms as a result of good hygiene practices, as other studies indicated that frequent cleaning of pens resulted in low accumulation of oocysts on farms.

The present study was the first epidemiological study conducted to determine the prevalence of *Cryptosporidium* sp. in pre and post-weaned dairy calves in Malaysia. The study demonstrated that the prevalence of *Cryptosporidium* in dairy calves was high. There was an association between different *Cryptosporidium* species and the age of the calves, with pre-weaned calves less than one month old being the major source of zoonotic *C. parvum.* Subtyping of the *C. parvum* isolates at the *gp60* locus revealed that the calves harboured zoonotic *C. parvum* subtypes (IIa and IId) and the finding of IId indicates unusual endemnicity in Malaysia. The result indicates that calves prior to weaning are a potential source of infection with this zoonotic parasite for farmers and veterinarians and for the general human population in the region through the contamination of food and water with oocysts.

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Table 1. Prevalence of *Cryptosporidium* in pre-weaned and post-weaned calves according to farming system.

Farming system	Pre-weaned calves		Post-weaned calves		Total
	No of positive calves	**Prevalence % (95% CI)**	**No of positive calves**	**Prevalence % (95% CI)**	
Intensive	**19/50**	38% (95% CI:24.5, 51.5%)	**19/70**	27.1% (95% CI:16.7, 37.6%)	**31.7%** (95% CI:23.3, 40%)
Semi-intensive	**18/70**	25.7% (95% CI:15.5, 36%)	**9/50**	18% (95% CI:7.4, 28.6%)	**22.5%** (95% CI:15, 30%)
Total	**37/120**	**30.8% (95% CI:22.6, 39.1%)**	**28/120**	**23.3% (95% CI:15.8, 30.9%)**	**27.1% (95% CI:21.5%, 32.7%)**

Table 2. *Cryptosporidium* species identified pre and post-weaned calves in Malaysia.

	No. typed	*C. parvum*	*C. bovis*	*C. andersoni*	*C. ryanae*	*Mixed spp.*
Preweaned	34/37	11 (32.4%)	9 (26.5%)	7 (20.6%)	4 (11.8%)	3 (8.8%)
Postweaned	20/28	0	7 (35%)	6 (30%)	6 (30%)	1 (5%)
Total	**54/65**	**11 (20.4%)**	**16 (29.6%)**	**13 (20.1%)**	**10 (18.5%)**	**4 (7.4%)**

Table 3. Results of binary logistic regression for management risk factors associated with *Cryptosporidium* infection in dairy calves.

Variables	***β* estimates**	**Odds Ratio (95% CI)**	**P- value**
Treatment given to the calves at least 3 times/year	-1.60	0.20 (0.08, 0.51)	0.001
Anthelmintics given to the calves at least 4 times/year	-2.03	0.13 (0.04, 0.43)	0.001
Pre-weaning pen with a slatted floor	1.93	6.88 (1.57, 30.18)	0.011
Other cattle farms less than 2.5 km away	2.04	7.72 (2.47, 24.17)	0.000
Post-weaning pen with a sand floor	1.50	4.50 (1.17, 17.31)	0.029
Feeding utensils washed with disinfectant	-1.50	0.22 (0.06, 0.80)	0.021
Calves fed saleable milk	1.95	7.04 (1.70, 29.20)	0.007
Constant	-2.02	0.13	0.004

Hosmer and Lemeshow statistic = 0.906, Cox and Snell R^2 value = 0.197, Nagelkerke R^2 value=0.286.

Table 4. Results from the univariable analyses of potential risk factors for C*ryptosporidium* infection.

Variables	Percent of samples positive for *Cryptosporidium* sp.	Odds Ratio (95% CI)	*P* value
Johor Bahru District	21.7	1.0	
Kluang District	32.5	1.7 (1.0, 3.1)	0.06*
Semi intensive farm	22.5	1.0	
Intensive farm	31.7	1.6 (0.9, 2.8)	0.11*
Female	22.1	1.0	
Male	31.5	1.6 (0.9, 2.9)	0.10*
Breed			
Jersey(reference)	10.0	1.0	0.06*
Mafriwal	15.9	1.7 (0.2, 15.6)	
Friesian crossbred	30.6	3.9 (0.5, 32.1)	
Post-weaned calves	23.1	1.0	
Pre-weaned calves	31.1	1.5 (0.8, 2.7)	0.17*
Calves not kept in a barn all the time	18.8	1.0	

Variables	Percent of samples positive for *Cryptosporidium* sp.	Odds Ratio (95% CI)	*P* value
Calves kept in a barn all the time	27.7	1.7 (0.5,6.0)	0.44
Calves not grazed at a certain time	27.7	1.0	
Calves grazed at a certain time	18.8	0.60 (0.17, 2.17)	0.44[#]
Calves not separated according to gender	28.2	1.0	
Calves separated according to gender	25.5	0.88 (0.49,1.56)	0.65
Calves not given ground water for drinking	24.7	1.0	
Calves given ground water for drinking	30.9	1.4 (0.8, 2.4)	0.29
Calves not given tap water for drinking	29.1	1.0	
Calves given tap water for drinking	25.4	0.8 (0.5, 1.5)	0.52
Water not treated before use	29.5	1.0	
Water treated before use	21.6	0.7 (0.3, 1.3)	0.20
Farms not practicing quarantine of new animals	27.4	1.0	
Farms practicing quarantine of new animals	26.8	0.9 (0.5, 1.7)	0.91
No other animals on the farm	22.5	1.0	
Other animals on the farm	29.8	1.5 (0.8, 2.7)	0.22
No treatment of faeces	31.2	1.0	
Treatment of faeces	13.0	0.3 (0.1, 0.8)	0.008[□]*

Variables	Percent of samples positive for *Cryptosporidium* sp.	Odds Ratio (95% CI)	*P* value
Calves not born in a multi-cow calving area	23.5	1.0	
Calves born in a multi-cow calving area	33.0	1.6 (0.9, 2.9)	0.11*
Calves not born in a single cow calving area	27.6	1.0	
Calves born in a single cow calving area	25.4	0.9(0.5, 1.8)	0.74
Calves born inside the yard	28.8	1.0	
Calves born outside the yard	23.8	0.8 (0.4, 1.4)	0.40
Calving area was not cleaned before calf birth	22.2	1.0	
Calving area was cleaned before calf birth	30.0	1.5 (0.8, 2.8)	0.19*
Calving pen without concrete floor	22.2	1.0	
Calving pen with a concrete floor	30.0	1.5 (0.5,2.8)	0.19#
Calves not separated from dam within 12 hours of birth	27.3	1.0	
Calves separated from dam within 12 hours of birth	27.0	1.0 (0.8, 1.7)	0.96
Pre-weaned calves not kept in common pens	5.0	1.0	
Pre-weaned calves were kept in common pens	29.1	7.8 (1.0, 59.5)	0.02□ *
Pre-weaned calves were not kept in individual pen	28.8	1.0	
Pre-weaned calves were kept in individual pen	23.4	0.8 (0.4,1.4)	0.37

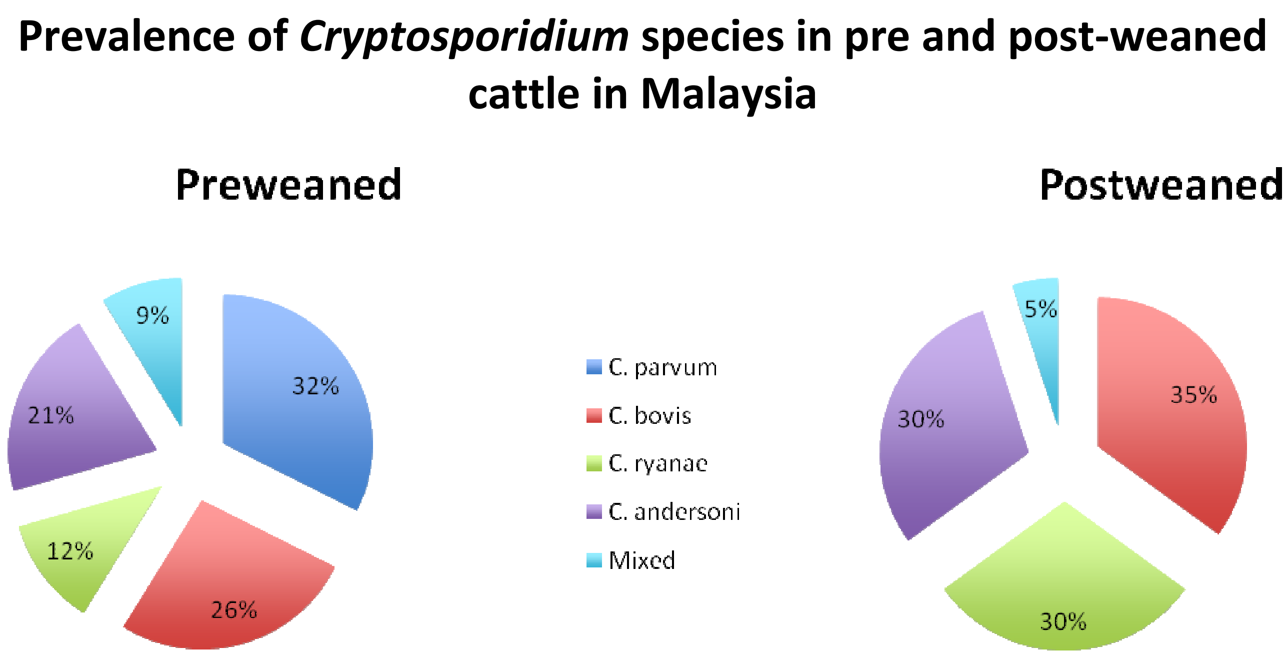
Variables	Percent of samples positive for *Cryptosporidium* sp.	Odds Ratio (95% CI)	*P* value
Pre-weaning pens without a concrete floor	25.8	1.0	
Pre-weaning pens with a concrete floor	28.0	1.1 (0.6,2.0)	0.71
Pre-weaning pens without a slatted floor	8.0	1.0	
Pre-weaning pens with a slatted flooring	32.1	5.4 (1.9, 15.8)	0.00□*
Pre-weaning pens without a sand floor	27.8	1.0	
Pre-weaning pens with a sand floor	10.0	0.3 (0.0, 2.3)	0.29
Calves get colostrum from another source	11.1	1.0	
Calves get colostrum from their mother	33.9	4.1 (1.8, 9.2)	0.00□*
Calves not given colostrum in a bottle	27.3	1.0	
Calves given colostrum in a bottle	27.0	1.0 (0.5, 1.8)	0.96
Calves not fed whole waste milk	28.6	1.0	
Calves fed whole waste milk	17.6	0.5 (0.2, 1.4)	0.18*
Calves not fed saleable milk	29.7	1.0	
Calves fed saleable milk	18.2	0.5 (0.3, 1.1)	0.09*
Calves not fed milk replacer	28.2	1.0	
Calves fed milk replacer	23.8	0.8 (0.4, 1.6)	0.50
Calves not receiving starter feed in the 1st week of life	22.6	1.0	

Variables	Percent of samples positive for *Cryptosporidium* sp.	Odds Ratio (95% CI)	*P* value
Calves receiving starter feed in the 1st week of life	35.8	1.9 (1.1, 3.4)	0.03□*
Feeding utensils not washed with disinfectant	20.8	1.0	
Feeding utensils washed with disinfectant	33.3	1.9 (1.1, 3.4)	0.03□*
Feeding utensils not washed with soap or detergent	26.8	1.0	
Feeding utensils washed with soap or detergent	27.6	1.0 (0.6, 1.9)	0.89
After weaning calves not kept in multi-age groups	25.2	1.0	
After weaning calves kept in multi-age groups	36.8	1.7 (0.8, 3.6)	0.14*
Post-weaning pens with other type of flooring	47.8	1.0	
Post-weaning pens with concrete flooring	24.9	0.4 (0.2, 0.9)	0.02#
Post-weaning pens with other type of flooring	24.9	1.0	
Post-weaning pens with sand flooring	47.8	2.8 (1.2, 6.6)	0.02□*
Herd not confined to an area surrounded by a fence	15.4	1.0	
Herd confined to an area surrounded by a fence	28.5	2.2 (0.8, 6.6)	0.24
Public or unauthorized personnel can't enter the farm	26.7	1.0	
Public or unauthorized personnel can enter the herd area	27.9	1.1 (0.7, 2.0)	0.85

Variables	Percent of samples positive for *Cryptosporidium* sp.	Odds Ratio (95% CI)	*P* value
No vehicle dip at the entrance of the farm	24.5	1.0	
Vehicle dip at the entrance of the farm	31.8	1.4 (0.6, 2.6)	0.23
No medication given to newborn calves	21.3	1.0	
Medication given to newborn calves	29.7	1.6 (0.8, 3.0)	0.18*
Mother was not treated for scours before calving	18.6	1.0	
Mother was treated for scours before calving	30.6	1.9 (1.0, 3.8)	0.06*
Calves faecal appearance normal	20.5	1.0	
Calves faecal appearance not normal	33.9	2.0 (1.1, 3.6)	0.02□*
Calves faecal appearance not fluid and watery	24.3	1.0	
Calves faecal appearance fluid and watery	61.1	4.9 (1.8, 13.2)	0.01□*
Calves faecal consistency normal	20.5	1.0	
Calves faecal consistency foamy, mucous and sticky	37.2	2.3(1.3, 4.1)	0.005□*
Calves faecal appearance not soft	26.2	1.0	
Calves faecal appearance soft	28.3	1.1 (0.6, 2.0)	0.73
Calves faecal colour green	25.7	1.0	
Calves faecal colour yellow, brown and grey	34.2	1.5 (0.7, 3.1)	0.28
Calves not infected with Giardia	26.7	1.0	

Variables	Percent of samples positive for *Cryptosporidium* sp.	Odds Ratio (95% CI)	*P* value
Calves infected with Giardia	30.0	1.2 (0.5, 2.7)	0.70

□-Variables associated with shedding of *Cryptosporidium* sp. in dairy calves (P < 0.05)
*-Variables offered to the logistic regression model
#-variables not selected because of high collinearity (correlation coefficient > 0.8)



Prevalence of *Cryptosporidium* species in pre and post-weaned cattle in Malaysia
Preweaned
Postweaned
32%
26%
12%
21%
9%
35%
30%
30%
5%
C. parvum
C. bovis
C. ryanae
C. andersoni
Mixed

Research Highlights

- First study in Malaysia to determine the prevalence, genotypes and management factors related to *Cryptosporidium* infections in pre-weaned and post-weaned dairy cattle
- Overall *Cryptosporidium* prevalence was 27.1%.
- *C. parvum* was most prevalent in pre-weaned and *C. bovis* was most prevalent in post-weaned calves
- gp60 subtyping identified IIdA15G1, IIa18A3R1 and IIa17G2R1.
- Having other cattle farms close by, feeding calves with saleable milk, keeping pre-weaned calves in pens with slatted floors increased the risk of *Cryptosporidium* infection