

PREVALENCE OF CRYPTOSPORIDIOSIS IN DAIRY CALVES IN CHENNAI

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ABSTRACT

The overall prevalence of Cryptosporidiosis in young calves in Chennai was 9.05 per cent. The incidence of cryptosporidiosis was highest (14.66%) in young calves in the age groups of 5-30 days followed by 31-60 days age group (9.38%), in older calves between the age group of above 3 months it was 6.06% and the lowest 3.23% in adult calves. Eighteen calves above 1 year were negative for Cryptosporidiosis. Analysis of data with regard to season wise prevalence indicated no significant difference in susceptibility. A significantly higher incidence of Cryptosporidiosis was observed in crossbred calves ($P>0.01$) than indigenous breeds. Females were more susceptible than male calves. In the present study it could be inferred that very young calves reared in unhygienic farm premises were highly susceptible to Cryptosporidial infections.

Key Words: Cryptosporidiosis, modified acid-fast staining technique, prevalence, young calves

INTRODUCTION

Cryptosporidium is an ubiquitous coccidian parasite that causes diarrhea in many mammalian species. It is the second most common pathogen from young calves with diarrhea (Hall *et al.*, 1992). Tyzzer (1907) published the initial reports of *Cryptosporidium* in mice. Anderson (1989) detected a low prevalence of Cryptosporidiosis in adult cattle and weaned calves for about 3.3% and 1.0% respectively. In 1993, Garcia and Lima reported 7 to 9% of Cryptosporidiosis in young calves less than 2 months old in Brazil. Nouri and Toroghi (1991) observed asymptomatic bovine *Cryptosporidium* to be primarily associated with 4 to 30 days old calves in Iran. Shobhamani and Alahasngari (2006) identified the prevalence of *Cryptosporidium* in calves in Andhra Pradesh. The

disease is characterized by low morbidity, which however may become severe when associated with other pathogens. *Cryptosporidium* sparked great Public health interest after the large human water borne outbreaks in Milwaukee in 1993 and rapidly was recognized as one of the most serious water borne pathogens to date. The diagnosis of Cryptosporidiosis relies on the identification of oocysts in faecal samples. As such there are only very few known reports on the prevalence and epidemiology of Cryptosporidiosis in India. Hence the present study was undertaken in order to understand the prevalence of Cryptosporidiosis in young ruminants in Chennai.

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MATERIALS AND METHODS

Animal source and geographic area

Twenty-five commercial small dairy herds were selected in Chennai. These small dairy herds were located at Elephant gate (3), Vyasarpadi (3), Mint (7), Walltax Road (6) and Triplicane (6). The small dairy herds consisted of 20-35 animals of both black and white cattle in a small confined area. The owners maintained their animals in the premises under unhygienic management without proper drainage facilities. The annual rainfall in these areas ranged from 0 to 20.6cm and environmental temperature ranged from 35 to 37^o C in summer, 30 to 35^o C in rainy and 29 to 33^o C in winter seasons. The animals were categorized as calves of less than 2 months old, calves above 2 months old, yearling cattle (12 to 24 months old), non-lactating and lactating cows and bulls on the day of sample collection.

Feed such as hotel waste, vegetable leftovers, rice and wheat bran, gram husk, groundnut husk for about 3 to 4kg / day / animal were provided by the owners. Among the feed, hotel waste and vegetable leftovers were provided ad libitum. The source of water for the animals was mainly from bore well and well water located near the coovum river.

Calves

Two hundred and twenty one dairy calves of both sexes and breeds were selected during March 2006 to April 2007 and followed up from birth to 4 months of age.

Collection of Faecal Samples

Two hundred and twenty one faecal samples (1 to 5g) from 25 small dairy herd calves with and without diarrhea were collected directly from the rectum of each calf, using a disposable latex glove. Samples were immediately placed in centrifuge tubes containing 5ml of 5% formalin in phosphate buffered saline solutions (PBSS, 0.9% NaCl, pH 7.2) and tubes were shaken vigorously

to disperse the faeces. Samples were stored at 4^o C until *Cryptosporidium* oocysts could be concentrated and enumerated. The faecal samples were clarified with 1M sucrose (Specific gravity, 1.13). Each faecal sample was then centrifuged at 1500 to 2000 rpm for 5 minutes in a fixed rotor centrifuge to concentrate parasite oocysts in the sediment.

The supernatant was decanted and the pellet was suspended in PBSS to a volume of 1ml. Each suspended faecal sample (25ul) was spotted on a clean glass microscope slide and the slide was air-dried for 30 minutes at room temperature. The slide was then fixed with acetone for 1 minute and left for drying (Ryan *et al.*, 1999).

Modified Acid –fast staining technique

The modified acid-fast staining technique was performed as per the described by Garcia *et al.*, (1983)

Microscopic examination

The stained smear was examined under high power objective. For confirmation of the internal morphology of the *Cryptosporidium* oocysts by 10 x 400 magnifications (Plate1).

Clinical signs

Calves were monitored during this study for signs of diarrhea and their age at onset and duration of each episode of diarrhea, during the first 4 months of life and were recorded.

Statistical analysis

The data were analyzed by chi-square test as per the method of Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

In the present study, the overall prevalence of Cryptosporidiosis in calves was found to be 9.05%. The present finding is in agreement with that of Garcia and Lima (1993) who reported

7-9% of bovine Cryptosporidiosis in young calves less than 2 months old in Brazil. Anderson (1989) detected 3.3% and 1.03% of Cryptosporidiosis in adult and weaned calves in USA. However, in our study it was detected in 3.23% adult calves, but in contrast, 6.06% was observed in weaned calves. The asymptomatic form noticed in the present study was in the age groups of 2-6 months; where as Nouri and Toroghi (1991) reported the asymptomatic form of *Cryptosporidium* in 4-30 days of age groups.

Shobhamani and Alahasingari (2006) reported that the prevalence of Cryptosporidiosis was highest (48.69%) in young calves in the age group of 31- 60 days followed by 1-30 days (43.24%) and lowest (13.09%) in adult calves. But in the present study the prevalence seemed to be less when compared to the above finding. The reason for the lower prevalence could be attributed to small sample size. The present study shows that the clinical evidence of Cryptosporidiosis was higher in less than 2 month of age (Table 1). This finding concurred with that of Olson *et al.*, (1995) who observed Cryptosporidiosis was high in 8 and 30 days of age groups. Ryan *et al.*, (1999) observed that 24% of calves of less than one month age groups were more susceptible than adult. However in our study relatively higher incidence (33.04%) was noticed. Underfeeding, poor hygienic environment etc. might be the reasons for such higher incidence.

According to Shobhamani and Alahasingari (2006) in Andhra Pradesh, indicated as overall prevalence of Cryptosporidiosis to be 31.80% in cross bred calves and 13.00% in indigenous breeds respectively. However, in our study a higher rate of prevalence was observed in crossbred calves (61.99%) and a slightly higher rate for indigenous calves (38.01%). The higher prevalence rate might be due to ill-hygienic management and environmental factors etc. Female calves were found to be more in this study due to the fact that they are retained and

male calves are disposed for slaughter, which is very common in Urban and rural places of the state (Table 2). The high prevalence rate was reported in male calves by Nouri and Toroghi (1991). The seasonal differences of Cryptosporidiosis observed were not marked and clinical disease could be noticed during all seasons. This finding concurred with Gopalnath (1997) (Table 3).

The highest prevalence of *Cryptosporidium* in a dairy herd could be attributed to poor management, high stocking rate (8-10 animals / 200 sq.ft). duration of calving seasons for cows in the herd increased from 0-60 days and up to year – around calving associated with wide range of ages, inadequate nutrition and exposure of young calves to heavily contaminated environment during calving seasons Mitchell *et al.*, 1997, Atwill *et al.*, 1999 and Nouri and Toroghi (1991), The above reference are in accordance with our finding in which the higher stocking rates / density (8 animals / 200 sq.ft), confined in closed proximity, lack of drainage facilities in the herds, increased duration of calving seasons in these dairy herds (0-80 days up to to year – around calving) might be the contributing factors to predispose the susceptible young calves to Cryptosporidial infections.

The clinical signs observed in the present investigation concurred with the reports of Casemore *et al.*, (1997), O'Donnoghue (1995) and Shobhamani and Alahasingari (2006) in which malabsorptive diarrhea, profuse watery diarrhea which is pale yellow in colour with a distinctive offensive smell and rarely blood tinged semi-solid faeces were recorded. However in our study, the *Cryptosporidium* oocysts were also detected (3.23%) in healthy calves. This finding is in agreement with Leek and Fayer (1984).

The diagnosis of *Cryptosporidium* oocysts was mainly based on the centrifugal sedimentation (Current and Garcia, 1991) and modified acid fast staining

methods (Garcia *et al.*, 1983 and Panciera *et al.*, 1971). The diagnosis of oocysts in the present study was based on the above methods and similar reproducible morphological characteristics were observed (plate 1).

ACKNOWLEDGEMENT

The authors wish to thank Tamil Nadu State Council For Science An Technology, DOTE Campus and the Dean, Madras Veterinary College, Chennai for providing grant and necessary facilities during the study period.

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Table 1:
Prevalence of Cryptosporidiosis in crossbred calves in Chennai during 2006 – 2007

Source of Samples	Age	Clinical Status	Samples		% Positive
			Tested	Positive	
Calves	5-30 days	Watery diarrhoea yellowish foetid odour	75	11	14.66
Calves	31-60 day	Watery diarrhoea mucous mixed along with dark tarry coloured faeces, blood tinged	33	6	9.38
Calves	3-6 months	Had diarrhoea one week before	33	2	6.06
Adult	7-12 months	Asymptomatic	31	1	3.23
Adult	12 months and above	Asymptomatic	18	--	--

Table 2:
Prevalence of Cyptosporidiosis in relation to breed and sex

Variables	Samples		% Positive
	Tested	% Positive	
Breed ($X^2 = 1.33$ $df=1$, $p<0.01$) indigenous crossbred	84	5	5.95
	137	15	10.95
Sex ($X^2 + 2.68$ $df = 1$, $p<0.01$) female Male	136	16	11.76
	85	4	4.71

Table 3:
Prevalence of Cryptosporidiosis in relation to season

Season	Samples		% Positive
	Tested	Positive	
Summer	74	7	9.46
Rainy	69	7	10.14
Winter	78	6	7.69

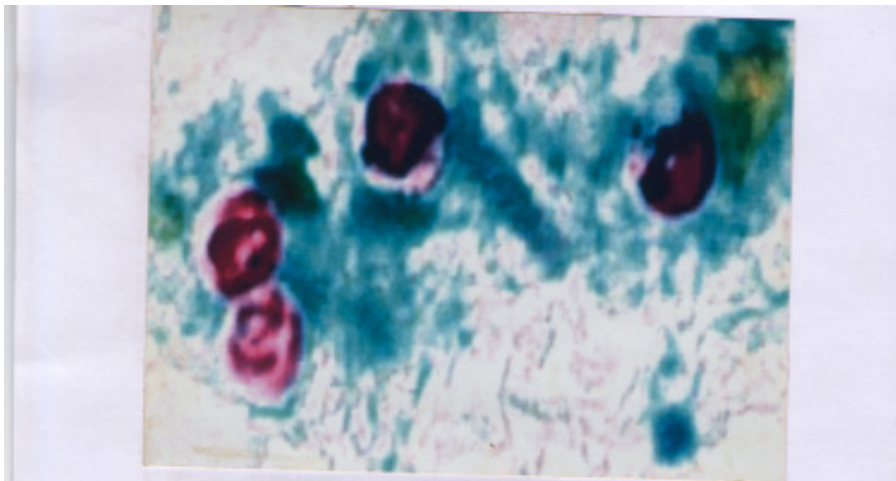


Plate 1: Bright red coloured oocysts of *Cryptosporidium* sp.
MAF x 400x