

AGE BUT NOT SEX IS A PRIMARY PREDISPOSING FACTOR FOR ASCARIOSIS IN BUFFALO CALVES: AN EVIDENCE FROM MUMBAI, INDIA

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ABSTRACT

Infections with GI nematodes often cause outbreaks of disease which is a major concern resulting in huge economic losses. There is vast economic impact of *T. vitulorum* and it mostly affects buffalo calves due to its survivability and causes heavy economic losses in India. In this study a total of 500 faecal samples of Murrah buffalo calves were examined having age below 3 months to know the status of *T. vitulorum* in Mumbai region. Out of 500 faecal samples 56 samples were positive for *Toxocara vitulorum*, Among the 6 age groups made, Group 1 (00 to 15 days), Group 2 (16 to 30days), Group 3 (31 to 45 days), Group 4 (46 to 60 days), Group 5 (61 to 70 days) and Group 5 (76 to 90 days) showed prevalence of *T. vitulorum*, 13.09%, 13.68%, 17.97%, 11.84%, 06.25% and 03.26% respectively. In sex wise prevalence of *T. vitulorum*, out 247 faecal samples of male buffalo calves, 33 (13.36%) found positive and out of 253 faecal samples of female buffalo calves 23 (09.09%) were found positive for *T. vitulorum* infection. The

EPG counts were found significantly increased during 1 to 2 months of age which subsequently decreases.

Keywords: *Bubalus bubalis*, buffaloes, age, Ascariosis, Mumbai, India

INTRODUCTION

Enteric and respiratory disorders have been studied by several workers as a major health disorder on calf mortality in India. Amongst the enteric disorders, diarrhea in the calves ranks topmost which causes great problem to the livestock owners because it causes stunted growth and increases the rate of mortality in calves (Yadav *et al.*, 2019). The *T. vitulorum* is among the most destructive parasites of calves, the larvae of which undergo migration causing great damage to many organs, especially the liver and the intestine. It is responsible for up to 50 % mortality and high morbidity in cattle and buffalo calves (Khatun *et al.*, 2009). The *T. vitulorum* mainly transmitted

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through transplacental and transmammary routes, causing the disease characterized by severe anaemia, diarrhoea, weight loss and anorexia, particularly in buffalo calves between 1 and 3 months of age (Wickramasinghe *et al.*, 2009). Toxocarosis can be diagnosed based on clinical signs, necropsy findings and faecal examination (Radiostits *et al.*, 2000).

Toxocarosis is a disease that mostly affects calf hoods up to six months of age. Adult worms are eliminated naturally, although the disease can cause substantial morbidity, productivity losses, and disruptions to breeding programmes (Deeba *et al.*, 2019; Mourya *et al.*, 2020). Pathogenicity caused by *T. vitulorum* infection in young calves is seen mainly in the form of diarrhoea and steatorrhoea. The primary pathogenicity resulting from a *T. vitulorum* infection in young calves is diarrhoea and steatorrhoea. Inappetance, intermittent colic, tympany, infrequent dysentery, anorexia, constipation, dehydration, abdominal pain, breath odour containing butyric acid, weight loss, and loss of plasma proteins in the intestine are associated with this infection (Roberts, 1993). Nonetheless, additional symptoms include skin tone and glossiness with eczema-like evidence of intestinal obstruction, the presence of foul-smelling, mud-colored faeces, lethargy, and recumbency are frequently observed (Abdel-Rahman *et al.*, 2015). The pathogenesis of infection can be more serious, and deaths can frequently be observed when associated with poor nutritional status (Nath *et al.*, 2016). Natural expulsion of adult worms commences as early as 38 days after birth and by 4 to 6 months no adult parasite remains in the intestine (Soulsbey, 1982). So, calf hood mortality due to heavy *T. vitulorum* infection is seen among buffalo calves of up to 6 months of age in India. If it is not controlled in the field, the prevalence can

go even up to 100% and the mortality rate can be as high as 80% (Bhangale, 2020). The *T. vitulorum* infection has been reported to have a cosmopolitan distribution in whole of the tropical and subtropical regions of the world with a moderate, continental climate. The prevalence of *T. vitulorum* in most of the countries is noted and reported between 5 to 50% (Dappawar *et al.*, 2020). The prevalence rate of this parasite is governed by species, sex, age, and seasonal variation (Bhangale, 2020).

Rearing a calf is crucial because it provides future replacement stock. The secret to productive and financially sustainable dairy production is optimised growth and minimal neonatal calf mortality. According to numerous studies, the small-holder buffalo production system's calves' poor health is caused by livestock owners' failure to promptly deworm their calves (Singh and Singh, 2000; Das, 2001). Because calves represent the dairy herd's future productive units, their survival is essential to the spread of animals. Considering the importance of this important nematode in buffalo husbandry and lack of data from the Mumbai region of Maharashtra state India, current study was conducted to apprise the prevalence status of *Toxocara vitulorum* worms in buffalo calves of the region.

MATERIALS AND METHODS

The coprological survey of buffalo calves from Mumbai area for detection of *Toxocara* spp. eggs was carried out during October 2020 to January 2021. Faecal samples were collected from 500 buffalo calves below 3 months of age belonging to 41 different buffalo farms present in the Mumbai region. Samples were divided into six groups according to age of buffalo calves by

making 15 days interval, such as Group 1 (0 to 15 days), Group 2 (16 to 30 days), Group 3 (31 to 45 days), Group 4 (46 to 60 days), Group 5 (61 to 75 days), Group 6 (76 to 90 days).

Approximately 10 grams of faecal sample from each buffalo calf was collected directly from rectum using plastic gloves and filled in plastic zip-lock bag. Samples were collected preferably in morning hours. The collected samples were examined grossly for colour, consistency, odour, parasites, and the presence of mucous, blood or any necrotic material. All the samples were brought to the laboratory immediately or shortly after collection. The samples were subjected to parasitological investigation on the day of collection itself. However, in case of inability to reach the laboratory on the day of collection itself, the samples were stored in 10% formalin as preservative.

In the laboratory, floatation technique for detection of helminthes ova/larvae was followed by using saturated Sodium Chloride solution as a floatation medium. The principle of floatation technique is based on specific gravity of salt used for floatation. When the worm eggs are suspended in a liquid with a specific gravity higher than that of eggs, the latter will float up to the surface (Soulsby, 1982). Subsequently, faecal samples of buffalo calves found positive for *Toxocara vitulorum* infection, were processed for the number of eggs per gram of the faeces to determine the severity of infection. Eggs per gram (EPG) of faecal samples were determined by Stoll's dilution method (Stoll, 1930).

RESULTS AND DISCUSSIONS

In the present study total 500 faecal

samples of Murrah buffalo calves having age below three months were collected to know the status of *T. vitulorum* infection in organised farms of Mumbai region. Out of 500 faecal samples, 121 samples were found positive for different G.I parasitic infections. Among 121 (24.20%) positive samples 56 (11.20%) samples were positive for *T. vitulorum*, 18 (3.60%) for *Strongyloides* spp., 14 (2.80%) for *strongyle* spp., 21(4.20%) for *Coccidia* spp. and 12 (2.40%) for *Trichuris* spp.

The age wise prevalence of *Toxocara vitulorum* was found highest prevalence in Group 3 (31 to 45days) which was 17.97% while least prevalence was found in Group 6 (76 to 90 days) as 3.26% (Table 2). However remaining groups, Group 1 (00 to 15days), Group 2 (16 to 30days), Group 4 (46 to 60days) and Group 5 (61 to 75 days) showed 13.09%, 13.68%, 11.84% and 6.25% prevalence of *T. vitulorum* respectively.

In accordance with our observations, Deeba *et al.* (2019); Zaman *et al.* (2014) also found the similar trend of infection and recorded prevalence was 18.54% and 17.75% prevalence rate of *T. vitulorum* respectively. Contrary to results of our study, Rao *et al.* (2000); Abdulalim aydi *et al.* (2005); Kaur *et al.* (2008) found relatively high *i.e.* 30.09%, 28.96% and 78.25% prevalence of *T. vitulorum* infection respectively. While other studies by Jyoti (2009); Maharana *et al.* (2016) recorded lower rate of infection as 8.47% and 5.26% in Punjab region and Junagadh respectively.

In the present study, the highest occurrence of *T. vitulorum* was noticed in calves of below one month age. These results are in accordance with the findings of Bharkad (1997); Biswa *et al.* (2021) who found higher prevalence in similar age group. During the study, buffalo calves aging 7 days were also observed to be positive for *Toxocara vitulorum* infection. Similar findings were observed by

Gupta (1986) and mentioned that it could be due to transplacental mode of infection. In the majority of buffalo calves the infection occurred after 10 days of birth reaching the peak after 30 days of birth. The highest prevalence was between 30 to 45 days which is attributed to transcolostoral infection.

Present data shows that infection reaches to peak between the 4th to 6th weeks of life. This indicates that infection has been derived through milk after birth. From statistical data it was found that infection peak was noticed around 36 to 38 days of life, which gives idea about the chances of infection through milk after birth much more than prenatal infection. Therefore, the control of *T. vitulorum* infection in young calves can be achieved by weaning of calf or deworming of calves within two to three days and thereafter up to two and half to 3 months.

The sex-wise distribution pattern of prevalence of *T. vitulorum* in buffalo calves revealed that out of 247 male samples collected found positive 32 (13.36%) and out of 253 female samples collected 23 (9.09%) found positive for *T. vitulorum* infection (Table 3). Rate of infection in male was more than female calves. According to earlier studies reported by Raza *et al.* (2013); Kebede (2018), genetic predisposition and differential susceptibility owing to hormonal control are two important factors which influence the susceptibility of animals to various infections. Similarly physiological status of female animals makes them weak. The present study noticed that the infection rate was more in male than female calves; this observation is an agreement with Bharkad (1997); Avcioglu and Balkaya (2011) who reported the comparatively higher infection in male than female calves. However, more burden of *T. vitulorum* in female than male animals were reported by Raza *et al.* (2013) and Kebede (2018).

The higher rate of infection in females might be due to lowered resistance of female animals on the part of their reproductive events and insufficient/unbalanced diet against higher needs (Mahieu *et al.*, 2008; Holzhauer *et al.*, 2010). Likewise, Das and Phukan (2018) also recorded the sex-wise prevalence higher (31.09%) in female calves than that male (20.31%) calves. Hamza *et al.* (2011) observed equal frequency of *Toxocara vitulorum* eggs in 60 (22.2%) male and 53 (22.3%) female calves. Prevalence of *T. vitulorum* did not differ significantly between the males and females. This could be the result of identical management systems offered to both sexes. Jyoti *et al.* (2012) made the same observations and found that there was no discernible difference in the prevalence of parasite diseases between the male and female buffalo calves in Punjab.

The second objective of study was to note the intensity of infection of *T. vitulorum* infection. For that EPG of faecal samples carried out which were positive for *T. vitulorum* infection and results were presented in Table 4 and Table 5. After processing the samples by Stoll's dilution method, it was found that range of EPG counts of 56 positive samples were 300 to 10800 and mean EPG was 3523.21±328.71. The highest average EPG was found in Group 3 (5943.75±692.70) following Group 2 (3438.46±458.05), Group 4 (2866.66±454.61), Group 1 (2309.09±243.26), Group 5 (1075±256.17), and least was in Group 6 (666.66±233.33).

The average estimated EPG in the present study in different groups of buffalo calves was 3523.21±328. Present study also revealed that the early evidence of higher EPG of *T. vitulorum* infection in buffalo calves was in 31 to 45 days age group, which explored the possibility of *T. vitulorum* infection, arrived during prenatal stage only. After 45 days, EPG count started to decline,

Table 1. Faecal sample collection sites from area around Mumbai.

Sr. no.	Sample collection area	No. of farms	No. of buffalo calves screened
1	Arrey Colony, Mumbai	16	184
2	Thane	11	178
3	Palghar	14	138
	Total	41	500

Table 2. Age wise distribution of *T. vitulorum* infection in buffalo calves at Mumbai.

Sr.no	Age groups	No. of faecal samples examined	No of positive samples	Percentage (%)
1	Group 1 (0-15 days)	84	11	13.09
2	Group 2 (16-30 days)	95	13	13.68
3	Group 3 (31-45days)	89	16	17.97
4	Group 4 (46-60 days)	76	9	11.84
5	Group 5 (61-75 days)	64	4	06.25
6	Group 6 (76-90 days)	92	3	03.26

χ^2 : 10.419 Non-significant.

Table 3. Sex wise distribution of *T. vitulorum* in buffalo calves ay Mumbai.

Age group	Male*			Female#		
	N	P	%	N	P	%
Group 1 (0-15 days)	40	7	17.50	44	4	09.09
Group 2 (16-30 days)	41	7	17.07	54	6	11.11
Group 3 (31-45days)	45	9	20	44	7	15.90
Group 4 (46-60 days)	40	6	15	36	3	08.33
Group 5 (61 – 75 days)	30	2	06.66	34	2	05.88
Group 6 (76 – 90 days)	51	2	03.92	41	1	02.43

N: Total sample checked; P: Number of positive samples; %: Percent prevalence.

* χ^2 : 6.321 and # χ^2 : 4.492 both non-significant.

Table 4. Age wise EPG of *T. vitulorum* in buffalo calves at Mumbai.

Age group	No. of samples positive	Range of EPG	Mean EPG± SE
Group 1 (0-15 days)	11	1200-3600	2309.09±243.26 ^b
Group 2 (16-30 days)	13	1400-6200	3438.46±458.05 ^{ab}
Group 3 (31-45days)	16	1800-10800	5943.75±692.70 ^a
Group 4 (46-60 days)	09	1000-5400	2866.67±454.61 ^b
Group 5 (61-75 days)	04	600-1800	1075.00±256.17 ^b
Group 6 (76-90 days)	03	300-1100	666.67±233.33 ^b

F-value: 9.53; F-Critical: 2.40 ($P \leq 0.05$) and 3.41 ($P \leq 0.01$).

Table 5. Sex wise EPG of *T. vitulorum* in buffalo calves at Mumbai.

Sr. No.	Sex	Mean ± SE	T Statistics	T Critical
1	Male	3681.25±409.87	0.54	2.00
2	Female	3325.00±532.30		

and it was found minimum in the age group 76 to 90 days having minimum EPG count 300 and the same group had 666.66 average EPG. The statistical comparison of EPGs between groups uncovered that the EPG of the calves in Group 3 (31 to 45 days) was found to be highest and it was significant at 5% and 1% level of significance than other age groups. But no statistically significant differences in EPG count of other groups were noticed. The peak of egg count for *T.vitulorum* was reported at 31 to 45 days of age in buffalo calves. These findings are similar with earlier studies of Bharkad (1997); Abdel-Rahman and El-Ashmawy (2013); Biswas *et al.* (2021).

The level of faecal egg count in buffalo calves indicates the worm burden level and decides the pathogenicity which has wide variation in

the opinions. It was ambiguously documented that the pathogenic EPG of *T. vitulorum* in calves may be as less as 200 (Lee, 1959) or even some reported it may be around 5000 (Lai and Canu, 1979); however these variations were attributed to absence of correlation between EPG and stage of infection and therefore decline in egg output was not due to loss of parasites but due to lower fecundity (Roberts, 1990). Although the higher EPGs has been suggestive of pathogenic worm burden, the clinical infections even with low EPGs cannot be ignored as very young calves may harbor numerous nematodes yet with low egg output (Bharkad, 1997; Rast *et al.*, 2013).

As regards the clinical symptoms corresponding with EPG count is concerned in present study it was observed that calves with

EPG higher than 1000 were having diarrhea, mild dehydration, and anorexia. However, calves with more than 5000 EPG showed symptoms as mucoid diarrhea, hyperthermia and intermittent tympany (Samal *et al.*, 2011; Ahmed *et al.*, 2016). These variations in results probably arose because the faecal egg count have not been related to the stage of infection.

The sex of the buffalo calves was taken into consideration when comparing the EPG counts of *T. vitulorum* in the calves. Regarding the calves' sex, statistically insignificant ($P \leq 0.05$) variations were noted. Comparison of EPG count in male buffalo calves and female calves is presented in Table 4.6. A mean \pm SE value of EPG count in male and female calves was found to be 3681.25 ± 409.87 and 3325.00 ± 532.30 respectively.

Magnitude or intensity of the infection varies from place to place, according to rearing system, according to temperature, rainfall, sex, and age group which was however partially evident as regards with the results recorded in present study. Prevalence of *T. vitulorum* was found to be higher in male buffalo calves than females but intensity of the infection was not statistically significant with respect to sex. It might be due to same rearing systems; same weather conditions were there for both the sexes which affected the results of EPG in males and female calves.

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