



## **Toxocara vitulorum in Faeces, Serum and Milk of Buffaloes in Giza Governorate**

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### **Abstract**

*Toxocara vitulorum*, a nematode parasite in the small intestine of cattle and water buffaloes, causes high morbidity and mortality of 1–3 months old buffalo calves. Absence of eggs in the faeces does not mean that animal free from infection. Coprological examination of random samples from 134 water buffaloes of different ages at Giza Governorate was compared with serological tests. It was found that the overall prevalence rate was 38 out of 134 (28.4%) using faecal examination. However, higher results were obtained (63.4%) using serological diagnosis in the same animals. The perienteric fluid antigen (Pe) of *T. vitulorum* was used to monitor the humeral immune-response by an indirect ELISA in a selected group of infected pregnant buffaloes and their newly born calves. *T. vitulorum* larvae were diagnosed in milk of infected lactating buffaloes during the first few days post parturition. Colostrum antibody concentration was highest on the first day post-parturition, but decreased sharply within 15 days. These antibodies were at highest concentration in the sera of calves from the first week and maintained at high level until 6 weeks of age, then started to decline reaching the lowest levels on week 20 after birth coincidentally with the rejection of the worms by the calves. The relation between shedding of the parasite eggs and increase in the age of infected calves were investigated. On the other hand, the relative number of eosinophil counts in the blood of naturally infected calves was investigated. It is concluded that, both humeral and cellular immune response may have an important role in *T. vitulorum* rejection by the calves and prevention of intestinal reinfection.

**Key words:** *Toxocara vitulorum*, Buffaloes, ELISA, Larva, Pe-antigen.

### **Introduction**

*Toxocara vitulorum* is a parasite of small intestine of ruminants, particularly buffalo calves of one to three months of age from tropical countries (Patnaik and Pande, 1963). It is responsible for high morbidity and mortality rates resulting in serious economic losses and zoonotic importance (Enyenihi, 1969). The adult worms of *T. vitulorum* are encountered principally in suckling calves. An adult female *T. vitulorum* produces thousands of eggs daily. Egg production ranges from 8000 to as high as 100,000 eggs per gram faeces per day. A thick protective shell provides resistance against adverse environmental conditions such as chemical and physical insult, enabling eggs to remain alive for many years (Roberts, 1989). The dam



sheds no *T. vitulorum* eggs even though she is the source of infection to the calves. This is because the larvae in the cow do not develop to adults but remain in third stage. When the cow is pregnant the larvae migrate from the liver to the mammary gland, and prior to parturition, to the milk through which the calf is infected (Roberts et al., 1990).

The diagnosis and control of *T. vitulorum* is not easy as the larvae migrate in the tissues, remaining as dormant or hypobiotic parasites. It is mainly transmitted through colostrum and milk, causing disease (severe anemia, diarrhoea, weight loss and anorexia) particularly in buffalo calves (Wickramasinghe et al., 2009). This parasite is of great problem of bovine and buffalo calves, particularly from poor tropical countries. Humans become infected by ingestion of infective eggs either from soil, dirty hands, raw fruits and vegetables or larvae from undercooked meat and unpasteurized milk (Borecka et al., 2010). Larval migration through different soft tissues in the human generates several clinical entities in the patient, such as visceral larva migrans, ocular toxocarosis, and neurotoxocarosis (Roldán et al., 2010).

Several serological tests such as IHA (Indirect Haemagglutination Test), CIEP (Counter-current Immuno Electrophoresis) and ELISA (Enzyme Linked Immune-Sorbent Assay) have been applied for immunodiagnosis of *T. vitulorum* (Singh et al., 2003) by using crude somatic antigens with variable results. Accordingly, the present study was aimed to investigate infection by *T. vitulorum* in buffaloes and their newly born calves during their first few months of life. The anti-*T. vitulorum* antibodies present in the sera and milk of infected buffaloes is evaluated by comparison with the level of parasite eggs in their faeces. In addition, the alteration in eosinophil counts was investigated at different times of infection.

## Materials and methods

### *Sampled animals*

A total of 134 water buffaloes of different ages from different regions of Giza governorate were used in this study. Samples were taken from 50 non pregnant animals, 30 calves' 6-8 month old and 34 calves (1-3 months of age) and 20 pregnant animals out of which 12 infected animals and their newly born calves were examined weekly till 6 weeks post-parturition, then after 12 and 24 weeks of age.

### *Collection of samples*

Faecal samples: -Rectal faecal samples were collected directly from rectum of each animal and examined by concentration flotation technique according to Soulsby (1968), faecal egg count was done by



McMaster counting technique according to Gordon and Whitlock (1939) and expressed in terms of eggs per gram of faeces (EPG).

Blood samples were collected in clean sterile dry screw capped tubes for making blood films and serum preparation for serological examinations. Blood smears were also prepared and stained with Giemsa stain for detection of eosinophils under oil immersion lens of microscope, according to Ferreira-Neto et al. (1981).

Colostrum/milk were collected in clean dry test tubes and rapidly frozen at  $-20^{\circ}\text{C}$ . Milk samples were divided into two parts; one part was examined on the day of parturition and daily for 15 days for the presence of *T. vitulorum* larvae according to Stoye (1976). The second part was prepared for serological examinations weekly for 6 weeks after birth according to Grundy et al. (1983). Serum and milk samples were aliquoted, labeled and stored at  $-20^{\circ}\text{C}$  till used.

#### *Preparation of antigen*

The Perienteric fluid (Pe) was collected from adult male and female parasites according to Amerasinghe et al. (1992). The protein content of the antigen was determined using the method of Lowry et al. (1951).

#### *Preparation of rabbit hyperimmune serum*

Rabbit anti-*T. vitulorum* anti-serum was prepared against the Perienteric (Pe) antigens according to Fagbemi et al. (1995), with slight modifications.

#### *Enzyme-Linked Immune-Sorbent Assay*

Indirect Enzyme-Linked Immune-Sorbent Assay (ELISA) was adopted to diagnose *T. vitulorum* among buffaloes using Pe- antigen. The assay was done as described by Santiago, et al. (1986) after little modification. The optimum antigen, serum and conjugate concentrations were determined by checkerboard titration. The Optical Density (Op.D) values were read at 405nm with micro-ELISA reader system (Titertek Multiscan, Flow laboratories, Mc lean, Virginia, USA). The tested sera were considered to be positive when the absorbance values were as or more than the cut off value (double of the mean of control negative samples).

## **Results**

The prevalence rates of *T. vitulorum* in buffaloes at Giza governorate via faecal and serological examination (ELISA) are illustrated in table 1.



*Faecal examination*

The results of faecal examination revealed that, out of 34 buffalo calves of 1-3 months 25(73.5%), out of 30 calves of 6-8 months 1(3.3%), no eggs were detected in any of 50 adults, 2-6 years and out of 20 pregnant buffalo cows 12(60%) were infected with *T. vitulorum*.

*Serological examination*

Perienteric antigen was selected to monitor the humeral immune response of buffalo cows and calves naturally infected with *T. vitulorum*. The results of serological examination (ELISA) using perienteric antigen (Pe-Ag) revealed that the prevalence rates were 27(79.4%), 14(46.7%), 26(52%) and 18(90%) for the same tested animals by faecal examination respectively.

**Table 1:- Prevalence of *T. vitulorum* in faeces and sera of naturally infected buffaloes at different ages.**

Host age	Number Examined	Faecal examination		Serological examination	
		(+ve)	(%)	(+ve)	(%)
Calves (1-3 months)	34	25	73.5%	27	79.4%
Calves (6-8 months)	30	1	3.3%	14	46.7%
Adults (2-6 years)	50	0	0%	26	52 %
Pregnant animal	20	12	60%	18	90 %
<b>Total</b>	134	38	28.4%	85	63.4%

**Table 2:- Larval recovery in the milk of *T. vitulorum* infected lactating buffaloes.**

Number examined	(+ve)	(%)	No. of larvae/100 ml. milk		Days of larval recovery	
			Mean	Range	Mean	Range
12	9	75%	5	2-9	7	3-12

*Buffalo calves*

The results of faecal examination from birth to 6 months of age demonstrated that the pre-patent period occurred approximately during the 3 weeks after birth and the maximum egg counts occurred during 6th week. After the peak, the number of eggs started decreasing in the faeces until disappeared completely after 20 weeks.

Antibody levels of buffalo calves naturally infected with *T. vitulorum* during their first year of life was assayed by ELISA with the results shown in Fig.1. At birth after suckling the colostrum, the antibody concentration was highest during approximately the first 45 days after the birth, the antibody levels maintained high, but thereafter the concentration of antibodies started to decline reaching the lowest levels on week 20. These antibody levels had remained stable later on until 6 months old.



*Milk examination*

An examination of milk samples from lactating buffaloes revealed that out of infected 12 animals, 9(75%) contain *T. vitulorum* larvae in their milk. The number of indicated larvae ranged from 2-9 with mean of 5 larvae per 100 ml. milk (table 2). The larvae were detected 3-12 with mean of 7 days post-parturition. Serological examination of milk (colostrum) from infected buffaloes revealed that antibodies concentration was the highest on the first few days of parturition but declined rapidly to reach very low concentration on 3rd week (fig. 2).

*Eosinophils in the blood*

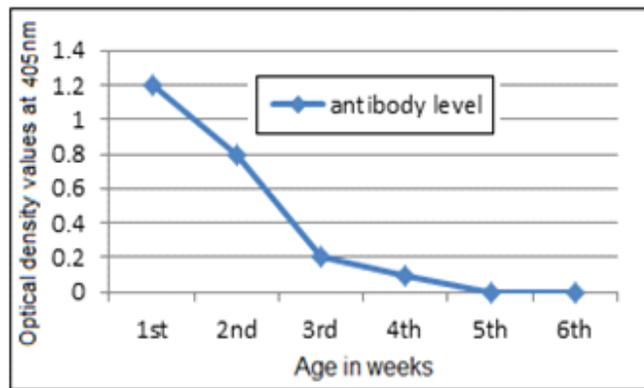


Fig. 1: Op.D values of *T.vitulorum* antibodies in the colostrum of buffalo cows during the periods from 1-6 weeks after birth.

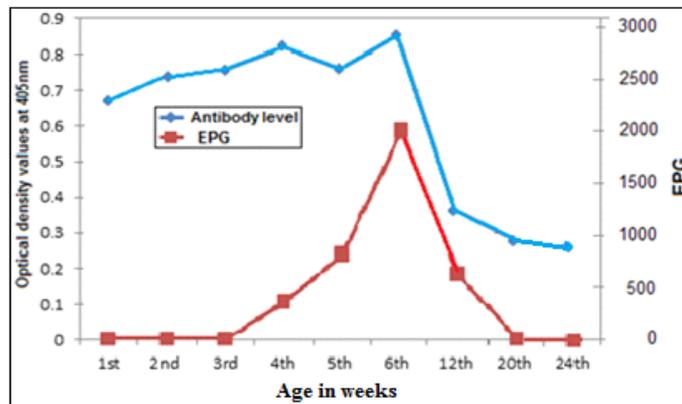


Fig. 2: Op.D values of *T. vitulorum* antibodies in the sera of buffalo calves during the period from 1-24 weeks after birth compared with the parasitic status of the calves represented by the EPG counts.

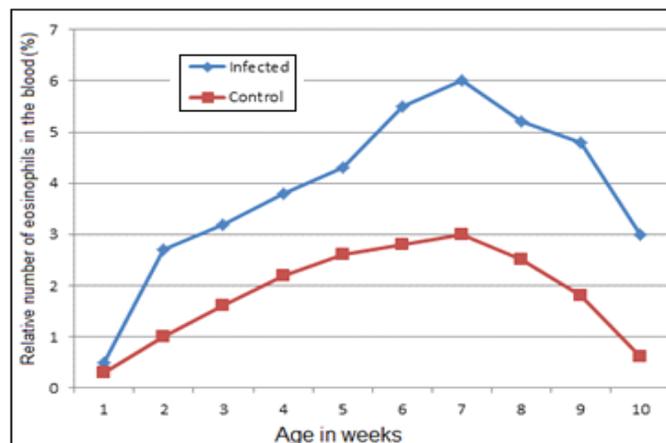


Fig. 3: Percentage of eosinophil's in blood of buffalo calves infected with *T. vitulorum* and control at the age of 1-10 weeks.

The results of the relative counts of eosinophils in blood stream of naturally infected and non-infected buffalo calves accomplished during 1- 10 week after birth days as shown in fig. 3. The mean eosinophil counts in the blood ranged from 1-3% in control and 1-6% in the infected calves. The number of eosinophil counts were very low for the first week, then increased after the second and remained at higher number between 2 and 7 weeks after birth, but then declined.

## Discussion

*Toxocara vitulorum* is a nematode parasite in the small intestine of cattle and water buffaloes, particularly buffalo calves 1–3 months old, causing high morbidity and mortality (Starke-Buzetti and Ferreira, 2005).

In the present study, the prevalence of *T. vitulorum* eggs in the faeces of buffaloes of different ages was lower than that recorded by serological diagnosis. The coprological diagnosis of *Toxocara* infection is not so accurate in latent infections in adult animals, where the larval stages are arrested in the tissues and in pre-patent and mild infections in young calves (Roberts, 2008). Accuracy of immunodiagnosis depends on the development of satisfactory extracts and successful choice of the assay. The selection of ELISA in the present research was based on previous studies suggesting ELISA as good tool for serodiagnosis of toxocariasis (Ghosh and Banerjee, 1998).

In the current study, the antigenic activity of the perienteric antigen was evaluated by ELISA in which hyperimmune serum was utilized. Souza et al. (2004) showed by ELISA that perienteric antigens (Pe) of *T. vitulorum* was more convenient than larval excretory/secretory (ES) antigens in terms of mass production and easy availability of the adult worms and highest levels of antibodies were detected by this antigen during the perinatal period.



The age susceptibility to *T. vitulorum* was observed in this study, where high infection rates were detected in young calves and very little or no infection in older calves and adult buffaloes (except pregnant and lactating animals). It is clearly evident that adult animals are refractory to the intestinal infection but under stress factors adult worms are developed in the intestine. Souza et al. (2004) found relationship between a marked immunosuppression due to stress of pregnancy and lactation and development of adult worms. Suppression of mitogen-induced lymphocyte and antibody titer decrease were reported also by Amerasinghe et al. (1994). On the other hand, the highest rate of infection (79.4%) in young calves (1-3 month), might be attributed to prenatal and postnatal routes of infection (from the infected mothers and the contaminated environment).

The current study indicated the presence of *T. vitulorum* antibodies in most calves more than 6 months of age and adult buffaloes although faecal examination was negative. According to Roberts (1989), the eggs of *T. vitulorum* are long-lived in the soil favoring a continuous infection of the pasture. But, the absence of eggs in faeces of these animals suggests that intestinal reinfection and development into adult worm did not occur. Similar results were obtained by Souza et al. (2004), they concluded that presence of resident migrating larvae in the tissues and possible acquisition of new infection by oral ingestion of infective eggs that might continuously stimulate the immune system of the old calves and adult buffaloes. Also, Ferreira and Starke-Buzetti (2005) stated that *T. vitulorum*-Pe antigen was able to protect calves against intestinal re-infection due to the allergen compounds of the Pe antigen that probably prevent adult worm to establish in the gut of older calves promoting an unsuitable environment for intestinal re-infection.

An examination of milk samples from lactating buffaloes revealed the presence of larvae which is identified as *T. vitulorum*, during 3-12 days post-parturition. Our results confirm that calves were exposed to infection from the first day of life as recorded by Omar and Lewis (1993). However, Roberts (1990) detected *T. vitulorum* larvae in the fetal liver and lungs of the pregnant buffaloes suggesting trans-placental or prenatal infection; although he concluded that the route of infection is mainly trans-mammary.

The public health importance of *T. vitulorum* larvae in milk was described by Glickman et al. (1987) who mentioned that diet such as unpasteurized milk and cheese obtained from infected animal act as risk factor associated with visceral larva migrans. Also, Magnaval (1992) mentioned that transmission of toxocariasis is common in children who habitually drink the colostrum early from dairy buffaloes.



While the buffalo calves received *T. vitulorum* larvae they also received specific IgG antibodies through colostrum as indicated by Souza et al. (2004). Serological examination of milk (colostrum) from infected buffaloes in the current study revealed that antibodies concentration was the highest on the first few days of parturition but declined rapidly to reach very low concentration at 3rd week. Roberts et al. (1990) detected *T. vitulorum* antibodies in the colostrum of buffaloes within 6 hours of parturition. On the other hand, anti-Pe antibodies were detected in serum of these calves after suckling colostrum suggesting passive transmission from mother serum through the colostrum. These results are in accord with the observation of Ferreira and Starke-Buzetti (2005) who suggested that anti-Pe antibodies of buffaloes were transmitted to calves through colostrum within 24 hours of birth. Also, Souza et al. (2004) detected high levels of antibodies against *T. vitulorum*-Pe antigen in the serum of buffalo cows and of actively suckling calves on the day of birth.

However, even buffalo calves receiving high serum levels of passively acquired antibodies against *T. vitulorum* on the first day after ingesting the colostrum, they developed patent infection. The buffalo calves in our study, started to shed *T. vitulorum* eggs through the faeces at 3rd week and peaked at 6th week post-parturition. The higher anti-Pe level antibodies, particularly in calves may be due to that it was obtained from adult worms as confirmed by Souza et al. (2004). They concluded that intestinal adult or juvenile worms may release antigenic molecules in the lumen of the intestine; and these molecules may stimulate the mucosal immune system.

Our studies indicated that a rapid decline of faecal EPG counts with the decrease of antibodies level in the serum of the calves after 6 weeks of age. This result suggests the expulsion of adult worms from the intestine and self-cure process. So, it is clear that passively acquired immunity does not protect the calves against the acquisition of the infection, but these antibodies, passively or actively acquired, may have an important role during worm rejection by the calves and prevention of intestinal reinfection.

In addition of humeral immune response, the present study demonstrated that buffaloes were able to mount a cellular mechanism. It is known that helminthes infection causes blood eosinophilia. The increase in eosinophil's occurred during the beginning and at the peak of the infection and then decrease during the rejection of the parasite. Apparently, the increased number of eosinophils in blood may migrate to the site of infection. Stevenson et al. (1994) stated that tissue eosinophilia has been also associated with the rejection of worm from the gut. It is found that eosinophil's release mediators that could attack nematode cuticle in the lumen of calf infection (Maria et al., 2003). Also, Jone's et al. (1994) suggested the role for such mediators and their cellular source in the expression of cellular immunity and worm rejection.



So it is possible to consider some protective mechanism which would reduce larval gut penetration and contribute to larval migration inhibition particularly to mammary gland of buffalo cows through useful immunization to avoid or reduce the potential transmission of the parasite to newly born calves through the colostrum. Also, it is recommended that deworming of young calves against *T. vitulorum* should be done 3-6 weeks of age in order to reduce dissemination of eggs, hence decreasing soil contamination with infected eggs. Treatment of milk from recently parturated buffalo before human consumption should be a matter of public health importance. Finally, early diagnosis via serological techniques would allow initiation of useful chemotherapy before extensive hepato-pulmonary larval migration with resulted pathologic changes, thus producing a significant impact on the economic aspect of the disease.

### **Conclusions**

We can recommend that, deworming of newly born calves against *T. vitulorum* infestation should be done between 3-6 weeks of age in order to reduce the rate of dissemination of eggs, hence decreasing the soil contamination with infected eggs. Treatment of colostrum/milk from recently parturated animals before human consumption should be considered as a matter of public health importance to avoid human infection with *T. vitulorum*. It worth mentioned that the level of antibodies as determined by ELISA, fecal egg count and eosinophil numbers coincide with each other and reach its peak at 6-7 weeks.

### **Conflict of interest statement**

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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