



Influence of natural infection of *Toxocara vitulorum* on markers of oxidative stress in Indian buffalo calves

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ABSTRACT

Present study envisages the possible oxidant/antioxidant imbalance due to toxocariasis in naturally infected buffalo calves before and after initiation of therapy. Buffalo calves (9) naturally infected with *Toxocara vitulorum* were selected to analyze hematological parameters, oxidative stress indices, levels of nitric oxide (NO) and minerals, viz. zinc, copper and iron and compared with 9 healthy buffalo calves. Pronounced reduction in the values of hemoglobin, packed cell volume, total erythrocyte count and oxidative stress indices like reduced glutathione, catalase and glutathione-s-transferase were observed in infected calves. However, significantly augmented levels of erythrocytic lipid peroxidation and serum NO were observed in infected calves. Levels of Zn, Cu and Fe significantly dropped in infected calves. All the infected animals were recovered completely after treatment with piperazine hydrate (300 mg/kg b. wt. PO for 2 days). Significant improvement in hematological parameters and mineral status were observed but no statistically significant improvement with the oxidative stress indices were observed in post treated calves. Results denote that incorporation of antioxidants in the therapeutic regimen might be helpful to curb the oxidative stress in the course of toxocariasis in buffalo calves.

Key words: Buffalo, Oxidative stress, *Toxocara Vitulorum*

Toxocara vitulorum (*T. vitulorum*) is the common ascarid parasite of *Bubalus* and *Bos* spp and affects animals worldwide (Avcioglu and Ibrahim 2011). In toxocarasis, a calf hood disease mostly up to 6 months of age, the adult worms are eliminated spontaneously (Roberts *et al.* 1990, Kassai 1999). *T. vitulorum* larvae settle in somatic cells and become activated after parturition of adult cattle and migrate to udder. Progeny calves contract infection through galactogenic transmission and develop into adult stage in intestine which passes eggs by 3 weeks causing diarrhea, poor performance, intestinal and biliary obstruction, and sometimes death (Srivastava 1963). Oxidative stress and reactive oxygen species (ROS) are incriminated at least in part to the development of pathogenesis due to worm infection (Schirmer *et al.* 1987). No single mechanism has been found responsible explicitly to define the pathogenesis. Certain studies suggested oxidative stress as one of the important mechanisms of parasitic pathology (Schirmer *et al.* 1987, Deger *et al.* 2008). Antioxidant defense system play a pivotal role in body to fight against excess free radical

generation (Stanner *et al.* 2004, Webb *et al.* 2008). Severe parasite infection cause anemia, chronic weight loss and even death (Mir *et al.* 2007). Altered hematinic and changing pattern of some enzymatic and non-enzymatic antioxidants were reported in parasitic diseases (Musaev and Elchiev 1983, Dede *et al.* 2002, Pabon *et al.* 2003, Mir *et al.* 2007) and probably no such information are available in Indian buffalo calves due to *T. vitulorum* infection. Therefore, the present study was designed to investigate any change in hematological, oxidant/antioxidant status and mineral profile in buffalo calves in the course of *T. vitulorum* infection.

MATERIALS AND METHODS

Buffalo calves irrespective of sex and breed, in the age group between 3 and 5 months presented at Referral Veterinary Polyclinic of the Institute with the signs of diarrhea, pot belly, palor mucus membrane, weakness, dull and depression were thoroughly examined and stool samples were collected to examine microscopically for the presence or absence of parasitic eggs. Buffalo calves (9) highly positive for *T. vitulorum* eggs during fecal examination received piperazine hydrate @ 300 mg/kg body weight orally once daily for 2 consecutive days (group 1) and no other medication was conferred. Another 9 healthy buffalo calves showing no diarrhea and negative for any parasitic eggs were

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considered as healthy control (group 2). All the animals were monitored and samples were collected before initiation of treatment (day 0) and thereafter on day 7 and day 15 after initiation of treatment.

Blood samples (5 ml) were collected for hematological parameters from each using ethylene diamine tetraacetic acid (EDTA, 1 mg/ ml of blood) as an anticoagulant and another 5 ml blood was collected in heparinized (1U/ml of blood) tubes. Blood was used within few hours for haematological investigation. Rest of the blood samples were utilized for separation of erythrocytes and plasma.

Hematological parameters, viz. hemoglobin (Hb %), packed cell volume (PCV %) and total erythrocyte count (TEC) were analyzed as per Jain (1986). Oxidative stress indices performed after preparing blood haemolysate as per standard procedure and used for different parameters. Erythrocytic LPO was estimated as per Placer *et al.* (1966). The nmol of malonaldehyde (MDA) / ml of erythrocyte hemolysate was derived using 1.56×10^5 /mol/cm as extinction coefficient (Utley *et al.* 1967). The GSH level in blood samples was determined according to Prins and Loos (1969). Reduced glutathione concentration in the test sample was calculated by using the molar extinction coefficient of dithio-bis-2-nitro benzoic acid (DTNB)-GSH conjugate 13600/M/Xcm. The CAT activity in haemolysate was estimated using H_2O_2 as substrate (Bergmayer 1983). The activity of enzyme was expressed in units/mg of hemoglobin. The GST activity was determined according to Habig *et al.* (1974). The specific activity of GST is expressed as mmol of GSH-CDNB (1-chloro-2,4-dinitrobenzene) conjugate formed/min/mg Hb (mU/mg Hb) using an extinction coefficient of 9.6/mM/cm. Nitric oxide level of blood plasma was measured by nitrate reduction on copper-cadmium alloy (Cu-Cd alloy) followed by color development with Griess reagent as per Sastry *et al.* (2002). Zinc, copper and iron concentrations in blood were estimated by atomic absorption spectrophotometer after acid digestion of the samples (Kolmer *et al.* 1951). Data were analyzed using paired 't' test and repeated measurement test with the help of statistical software package SPSS 10.0. The levels of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Fecal examination of infected calves revealed presence of *T. vitulorum* eggs. Comparison of mean values of hematological indicators between 2 groups is presented in Table 1. There was significantly ($P < 0.05$) reduced levels of Hb, PCV and TEC in *T. vitulorum* infected calves (group 1) as compared to healthy calves (group 2) before instituting therapy and all the hematological parameters revealed significant ($P < 0.05$) elevation by day 7 after therapy with piperazine hydrate. However, the values on day 7 and day 14 after treatment did not differ significantly. Among oxidative stress markers, erythrocytic LPO and serum NO depicted significant ($P < 0.05$) increased level whereas, GSH,

CAT and GST revealed significant ($P < 0.05$) decreased level in infected calves before initiation of treatment as compared to healthy calves. Altered levels of the oxidative stress indices and NO level remain unaffected till the day 14 of treatment and institution of therapy with anthelmintic did not affect in the values (Table 1). Significantly ($P < 0.05$) decreased levels of Zn, Cu and Fe were observed in the infected calves than healthy calves which remain unchanged till day 7 post treatment, however, significant improvement of mineral levels were noticed on day 15 after initiation of therapy. After treatment, all the infected animals recovered completely based on fecal examination without any side effects. No significant variation observed in the blood parameters of the control calves throughout the study period. Infection of *T. vitulorum* in cattle and buffalo calves causing morbidity and mortality has been described from time to time (Roberts *et al.* 1990, Kassai 1999, Hassan and Aziz 2010). The present result corroborates with the findings of Hayat *et al.* (1999). In the present investigation, anemia might be attributed to depressed erythropoietic activity of bone marrow and /or depletion of nutrient due to inadequate absorption from gastrointestinal tract.

The level of Zn, Cu and Fe significantly ($P < 0.05$) decreased in infected calves compared to healthy calves. It seems that overall utilization or sequestration of zinc and copper to neutralize the overproduction of reactive oxygen species (ROS) might be responsible for the poor mineral status in blood of the infected calves of the present study. Significant ($P < 0.05$) improvement in the values of hematology and mineral profile by day 15 of post treatment with piperazine hydrate, might be due to restoration of normal nutrient absorption of intestine and initiation of erythropoiesis as a consequence of decreased level of toxin level caused by *T. vitulorum* (Hayat *et al.* 1999). Marked mineral deficiency along with decreased levels of erythrocytic parameters (PCV, Hb and RBC value) and macrocytic hypochromic anemia in ascariasis of buffalo heifers was reported by El-Moghazy (2011). He also noticed in buffaloes, a strong negative relationship between parasitic infestation and oxidative stress of the animals.

This was the first study to explore the relationship between *T. vitulorum* infection and oxidative stress, which is a well-established mechanism of cellular injury in buffalo calves. In the present investigation, erythrocytic LPO significantly ($P < 0.05$) augmented whereas, GSH, CAT and GST were significantly ($P < 0.05$) decreased in infected calves as compared to healthy calves before initiation treatment. Our results strongly suggested that *T. vitulorum* infection induces oxidative damage and depletes antioxidant system in buffalo calves. Deger *et al.* (2008) observed that the concentration of MDA was high, but the activities of SOD and CAT and the concentration of GSH, vitamin C and beta-carotene were low in cattle infected with gastrointestinal nematode. Similarly, other researchers also found elevated oxidative

Table 1. Hematology, oxidative stress indices and mineral profile in ascariasis infected (group 1) and control (group 2) calves at pre and post therapy periods

Parameters	Groups	day 0	day 7	day 15
<i>Hematology</i>				
Hb (g %)	1	6.21±0.36 ^{a,A}	9.57±0.27 ^{b,A}	10.61±0.44 ^{b,A}
	2	12.33±0.46 ^{a,B}	12.09±0.46 ^{a,B}	12.22±0.35 ^{a,A}
PCV (%)	1	26.33±1.19 ^{a,A}	33.55±1.33 ^{b,A}	35.77±1.15 ^{b,A}
	2	41.00±1.10 ^{a,B}	41.00±1.47 ^{a,B}	40.22±1.32 ^{a,B}
TEC (×10 ⁶ /cmm)	1	3.12±0.17 ^{a,A}	5.05±0.16 ^{b,A}	5.42±0.11 ^{b,A}
	2	6.25±0.15 ^{a,B}	6.11±0.14 ^{a,B}	6.18±0.17 ^{a,B}
<i>Oxidative stress indices</i>				
LPO (nmolMDA/ mg Hb)	1	4.62±0.61 ^{a,A}	4.48±.59 ^{a,A}	4.52±0.69 ^{a,A}
	2	2.09±0.18 ^{a,B}	2.21±0.19 ^{a,B}	2.15±0.18 ^{a,B}
GSH (Units/mg Hb)	1	2.06±0.23 ^{a,A}	2.01±0.22 ^{a,A}	2.06±0.21 ^{a,A}
CAT (K/ mg Hb)	2	5.61±0.24 ^{a,B}	5.60±0.23 ^{a,B}	5.61±0.22 ^{a,B}
	1	2.68±0.13 ^{a,A}	2.62±0.13 ^{a,A}	2.65±0.12 ^{a,A}
	2	5.18±0.20 ^{a,B}	5.15±0.21 ^{a,B}	5.18±0.21 ^{a,B}
GST (U/mg Hb)	1	0.15±0.01 ^{a,A}	0.15±0.01 ^{a,A}	0.16±0.01 ^{a,A}
	2	0.70±0.05 ^{a,B}	0.70±0.05 ^{a,B}	0.71±0.05 ^{a,B}
NO (µM/ml)	1	27.77±2.52 ^{a,A}	26.55±2.47 ^{a,A}	25.33±2.59 ^{a,A}
	2	15.65±1.06 ^{a,B}	15.39±1.53 ^{a,B}	15.64±1.55 ^{a,B}
<i>Mineral profile</i>				
Cu (ppm)	1	0.43±0.06 ^{a,A}	0.44±0.06 ^{a,A}	0.56±0.05 ^{b,A}
	2	0.79±0.03 ^{a,B}	0.80±0.03 ^{a,B}	0.81±0.03 ^{a,B}
Zn (ppm)	1	0.39±0.02 ^{a,A}	0.40±0.02 ^{a,A}	0.51±0.02 ^{b,A}
	2	0.60±0.02 ^{a,B}	0.61±0.02 ^{a,B}	0.63±0.01 ^{a,B}
Fe (ppm)	1	0.055±0.006 ^{a,A}	0.055±0.007 ^{a,A}	0.063±0.006 ^{b,A}
	2	0.086±0.002 ^{a,B}	0.087±0.002 ^{a,B}	0.087±0.002 ^{a,B}

Values with different superscripts differ significantly (P<0.05) from day 0 of treatment; values with superscripts (A, B) differ significantly (P<0.05) between groups.

stress in human patients with *A. lumbricoides* infection and they further confirmed that the occurrence of oxidative stress and lipid peroxidation as a mechanism of tissue damage in *A. lumbricoides* infection (Kilic *et al.* 2003, Chandramathi *et al.* 2009).

Nitric oxide (NO) is implicated as an integral component of the host defender against invading parasites. Effect of NO in pathogenesis of parasite infections were extensively studied in laboratory animal models (Demirci *et al.* 2006, Kolodziej-Sobocinska 2006, Donji *et al.* 2008). Many pro-inflammatory cytokines stimulates the synthesis of NO, which mediates host protection through either restricting parasite growth or by direct parasite killing (Youfang *et al.* 2008). Gargili *et al.* (2004) observed NO mediated brain tissue damage in mice experimentally infected with *Toxocara canis*. In the present investigation, a pronounced increase of plasma NO in infected calves might be due to cytokine production from the stimulatory effect of secretory/excretory toxins of *T. vitulorum*. Based on current finding, it could be hypothesized that *T. vitulorum* infection has a significant influence to augment NO production and during pathogenesis of toxocarasis, excess free radicals lead to the occurrence of oxidative stress. Administration of anthelmintic alone

(piperazine hydrate) did not reduce the oxidative stress as evident from various stress parameters (LPO, GSH, CAT, GST and NO) even on day 15 of post treatment, which clearly indicate that incorporation of antioxidant in the therapeutic regimen might be an alternate choice to alleviate the condition. Concluding the present study indicated a possible relationship between oxidant/antioxidant imbalances in *T. vitulorum* infection of buffalo calves though further detailed studies are required to establish actual mechanism of pathogenesis. Such work suggests that incorporation of antioxidants in the therapeutic regimen might be helpful to curb the oxidative stress in the course of toxocarasis in buffalo calves.

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