

13. Egypt. J. Med. Sci. 37 (2) December 2016: 719-726.

CLINICAL AND THERAPEUTIC INVESTIGATION ON *TOXOCARA VITULORUM* INFESTATION IN BUFFALO CALVES

By

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ABSTRACT

Gastrointestinal parasites in production animals are of economic value, therefore, treatment warranted to keep high profit. As little is known about use of different drugs on ascariasis in buffalo, the goal of the present study was to characterize the clinical findings and treatment outcomes of toxocariasis in buffaloes in Delta region, Egypt. A total of 204 buffaloes of different ages and sex were examined for the presence of *Toxocara vitulorum* over a period of 6 months at Delta region, Egypt. *Toxocara vitulorum* was identified in 16.6 % of the examined buffaloes. The disease was more prevalent in calves less than 6 months. The clinical reactions were varied from nil to severe. This was differs according to the animal age and the severity of infestation. These signs were inappetence, diarrhea or constipation, slight tympany and pica in most cases. Normal body temperature was observed in all infected cases. Concerning the haematological findings, the haemoglobin levels, packed cell volume and total

erythrocyte count were lower in ascarid-infected calves than in controls. on other hand, eosinophilia was observed in infected calves compared to control ones. Evaluation of ivermectin, and piperazine citrate was assessed in these naturally infected animals. Deworming of the host by the anthelmintic led to normalization of the clinical and hematological parameters after two and four weeks post-treatment for ivermectin and piperazine citrate, respectively.

INTRODUCTION

Parasitic infestations are worldwide problem, particularly in the third world countries. In animals, the infestation usually results in serious economic losses due to mortality, morbidity and/or decrease of performance (Avcioglu and Balkaya, 2011; Borgsteede et al., 2012; Chelladurai et al., 2015; Li et al., 2016).

Toxocara vitulorum is a cosmopolitan large intestinal nematode frequently associated with heavy morbidity and mortality in buffalo calves (Roberts, 1989). It was considered a highly preva-

lent parasite in 15-50-day-old water buffaloes (Starke et al., 1996). Adult animals acquire larval infection from infective eggs in wallow water, soil and pasture, while young buffalo calves become infected mainly by ingesting infective larvae from the colostrum of their mothers during the first days after the birth (Roberts et al., 1989).

Toxocara vitulorum larvae penetrate the wall of the small intestine between 2 and 8 h after ingestion. Most larvae go straight to the liver via the portal vein but a few enter the mesenteric lymph nodes. Over the next 90 h some larvae migrate to the lung and a few to muscles, brain, kidney and peripheral lymph nodes. Most remain in the liver. Over the next 3–7 weeks the larvae grow by about 10% and no moulting is observed. In a pregnant host, larvae grow to 500–600 μm in liver and lung 1–8 days before parturition and migrate to the mammary gland around the time of parturition. In the mammary gland they grow to about 1200 μm and pass into the milk during the 7 days after parturition (Roberts, 1990; Lawrence, 2003). The first detection of *T. vitulorum* eggs in buffalo calf feces occurred on day 11, confirming the very short pre-patent period also reported (Roberts 1990; Starke et al. 1996).

The adult parasites are relatively easy to remove from the intestines by chemotherapy, while the larvae are difficult to be killed mainly when they are in the musculature and in the brain (Ribeiro et al., 2000).

Attention was drawn to the lack of scientific evidence for the effectiveness of many anthelmintics on the parasite, especially in buffalo calves. Consequent-

ly, randomized clinical trial was conducted to assess the efficacy of ivermectin and piprazine citrate in the treatment of *Toxocara vitulorum* infestation in buffalo calves..

MATERIALS AND METHODS

Animals

A total of 204 buffaloes of different ages and sex were examined for the presence of *Toxocara vitulorum* over a period of 6 months.

Clinical examination:

Complete clinical history and physical examination for each animal were carried out according to Radostits et al. (2007).

Parasitological examination:

Fecal samples were collected directly from the rectum of the examined animals, prepared for examination by floatation technique (Kelly, 1984). In treated animals, fecal samples were examined on days 7, 14, 21, 28 and 56 post-treatments to ensure effective therapy (Ihler, 1995).

Hematological examination:

Blood samples were collected from each animal via jugular vein puncture to perform complete hematological examination. Blood samples were taken at the day zero of treatment and at 7, 14, 21, 28 and 56 days post treatment.

Therapeutic trials:

Sixty *Toxocara vitulorum* infected calves were allocated randomly into three groups (20 buffalo each). The first group received one dose of ivermectin at a dose rate of 200 $\mu\text{g kg}^{-1}$ subcutaneously. However, the third group received pipra-

zine citrate orally at a dose rate of 300mg kg⁻¹. The efficiency of each regime was evaluated on the basis of clinical, hematological and parasitological response which was studied on day zero of treatment and on days 7, 14, 21, 28 and 56 post treatments.

Statistical analysis

Statistical analysis was carried out by using statistical software program (SPSS for windows, version 16, NC, USA). Treatment outcomes were assessed firstly by evaluation of homogeneity of groups of buffalo calves, the fecal egg counts on the day of the first examination were compared between groups by the Man wintry nonparametric test. Furthermore, data was subjected to repeated measures ANOVA to determine the main effect of drugs and time. The Walk's Lambda test was selected to evaluate within group interactions and evidence of time×drug interactions. Where Walk's Lambda test indicated a statistically significant difference between drug groups, t- test was used to identify which group was statistically different from the rest. Differences between means at P<0.05 were considered significant.

RESULTS

Concerning the disease prevalence, out of the examined 204 buffaloes of different ages and sex, 34 (16.6%) animals proved to be infected. Concerning the age predisposition, the prevalence of the disease was more prevalent in calves less than 6 months (20 animals), followed by animals over 6 months and less than 1 year (9 animals) and finally the animals over than 1 year (5 animals).

Concerning the clinical signs, the clinical reactions were varied from nil to

severe. This was differs according to the animal age and the severity of infestation. All the infected animals showed normal body temperature. Adults always showed no clinical signs. Calves between 6 months and 1 year showed mild clinical signs in the form inappetance and retarded growth rates. Calves younger than 6 months were severely affected. Inappetence was the most prominent in these infected calves. Diarrhea was observed in 22 out of them. Constipation with dark discoloration of feces and slight tympany were observed in 12 out of them. Pica was observed in 34 calves.

Concerning the haematological findings (Table, 1), the haemoglobin levels, packed cell volume and total erythrocyte count were lower in ascarid-infected calves than in controls.

After treatment with ivermectin and piprazine, diarrhea, distention and inappetence of all treated groups were completely disappeared within the 1st. week after treatment. Moreover, pica was remained till the 4th week. The recovery rates were 36% and 70% in the 1st. week and 70% and 80% in the 2nd week while the rates were 80% and 90% in the 4th week for, ivermectin and piprazine, respectively. Whereas these rates reached 100% recovery rate in the 8 weeks in all the treated groups.

Efficacy of drug was assessed on the basis of reduction in egg per gram (Epg) and disappearance of clinical signs. Treatment trial proved that piprazine citrate gave the better results compared with ivermectin.

On parasitological level, the results of the present investigation showed that significant decrease of the fecal egg

count was recorded by the time (repeated measures test, $P < 0.05$). There were also significant differences between the effects of drugs. Thus, complete clinical and parasitological cure were occurred, by the fourth week in ivermectin and piperazine treated group (Table 2)

Deworming of the host by the anthelmintic led to normalization of the hematological parameters after 4 weeks

in case of ivermectin and piperazine citrate (Table 3).

Concerning the adverse effect of the used drugs, the clinical observations after treatment revealed that there was an adverse effect after use of piperazine citrate represented by diarrhea and restlessness. However, use of Ivermectin didn't show any abnormal clinical signs.

Table (1): Haemogram in healthy and *Toxocara vitilourum* infected buffaloes (Mean \pm SD).

Variable	Healthy buffaloes (N=10)	Diseased untreated buffaloes (Early stage) (N=34)
RBCs ($10^6/\mu\text{l}$)	10.31 \pm 0.02	8.20 \pm 0.23
Hb g/dl	13.55 \pm 0.17	9.99 \pm 0.26
PCV %	37.29 \pm 1.7	21.08 \pm 3.5
WBCs ($10^3/\mu\text{l}$)	8.75 \pm 0.30	8.57 \pm 0.41
Neutrophils ($10^3/\mu\text{l}$)	2.90 \pm 0.31	2.30 \pm 0.31
Lymphocytes ($10^3/\mu\text{l}$)	3.82 \pm 0.10	3.82 \pm 0.13
Monocytes ($10^3/\mu\text{l}$)	0.39 \pm 0.02	0.36 \pm 0.04
Eosinophils ($10^3/\mu\text{l}$)	0.17 \pm 0.02	0.19 \pm 0.03
Basophils ($10^3/\mu\text{l}$)	0.01 \pm 0.002	0.01 \pm 0.003
Thrombocytes ($10^3/\mu\text{l}$)	250.18 \pm 32.4	198.37 \pm 30.56

RBC, red blood cells; WBC, white blood cells; Hb, haemoglobin concentration; PCV, packed cell volume.

Table (2): Effect of ivermectin and piperazine citrate on the fecal egg count of *Toxocara vitillorum*.

Drug	Time post-treatment (week)				
	Zero	1	2	4	8
Ivermectin	19.000	1.500	1500	1200	1100
Piperazine citrate	21.000	2000	2000	1000	650

Meian with different superscript letter in the same column are significantly different at $P < 0.05$

MANOVA fit, $P < 0.018$

Wilks' Lambda test for drug x time interaction, $P < 0.05$

Table (3): Effect of ivermectin and piperazine citrate on the esinophils in calves infested with *Toxocara vitillorum*.

Drug	Time post-treatment (week)				
	Zero	1	2	4	8
Ivermectin	2498 ± 78	1859 ± 58 ^b	1398 ± 49 ^b	1214 ± 57 ^b	1129 ± 36 ^b
Piperazine citrate	2436 ± 93	1388 ± 37 ^a	864 ± 27 ^a	612 ± 65 ^a	620 ± 24 ^a

Means with different superscript letter in the same column are significantly different at $P < 0.05$

MANOVA fit, $P < 0.018$

Wilks' Lambda test for drug x time interaction, $P < 0.05$

DISCUSSION

In tropical countries, toxocariasis is one of the most serious parasitic diseases causing high mortality and morbidity in buffalo calves (Srivastava and Sharma, 1981). Even, the adult buffalo is the source of infection; the suckling calves play the potential role for maintenance the infection. As the larvae do not develop to adults into the adult animal, but remains as third- stage larvae and when the infected dams become pregnant, the larvae migrate from the liver to the mammary gland, and sucking calves receive the infection into the milk (Roberts et al., 1990).

Concerning the disease prevalence, 16.6% of buffalo proved to be infected. Higher prevalence was recorded by Maqbool et al. (1998) 26.66%. Lower prevalence was recorded by Halmandge et al. (2006) (9%). Regarding the age predisposition, the prevalence of the disease was more prevalent in calves less than 6 months. Similar observation was recorded by Maqbool et al. (1998), islam et al. (2005), Halmandge et al. (2006).

Clinically, the clinical reactions were varied from nil to severe. This was differs according to the animal age and the severity of infestation. Similar signs were observed in previous study (Rajkhowa et al. 2004). In contrast, Islam et al. (2005) observed only diarrhea in a percentage of 100% of the infected calves. The constipation recorded in our study may be revealed to the obstructive mechanism by the presence of massive number of the parasite.

Concerning the haematological findings, similar findings were recorded by Rajkhowa et al. (2004). On other hand, except for eosinophils, there were no significant haematological changes in buffaloes infected with *T. vitulorum*.

Diarrhea, distention and inappetence of all treated groups were completely disappeared within the 1st. week after treatment. Moreover, pica was remained till the 4th week. The recovery rate was 80% and 90% in the 4th week post-treatment for ivermectin and piperazine respectively. Whereas these rates reached 100% recovery rate in the 8

weeks in all the treated groups.

Efficacy of drug was assessed on the basis of reduction in egg per gram (Epg) and disappearance of clinical signs. Treatment trial proved that piperazine citrate gave the best results compared with ivermectin. On the contrary, Kumar and Ram Rao (2000) found that piperazine, ivermectin and levamisol showed 100% efficacy.

Deworming of the host by the anthelmintic led to normalization of the hematological parameters in piperazine citrate earlier than ivermectin treated group. This indicates a firm association between the recorded results and the presence of the parasite. Similar results were recorded by Rajkhowa et al. (2004).

Concerning the adverse effect of the used drugs, the clinical observations after treatment revealed that there was an adverse effect after use of piperazine citrate represented by diarrhea and restlessness. Adult worms are known to be more susceptible to the action of piperazine and larval stages in host tissues, especially larvae that are moulting, are little affected by the drug (Islam et al., 2006). Piperazine was associated with some side effects like diarrhea and restlessness (Akhtar et al., 1982). In conclusion, piperazine citrate produced more rapid and better effect on *Toxocara vitulorum* infestation in buffalo calves.

Conflict of interest

Authors declare that there is no conflict of interest

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دراسة اكلينيكية وعلاجه على العدوى بالتكسوكارا فيتولوروم فى علاج الجاموس

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صبرى احمد الخضرى

قسم الباثولوجيا الاكلينيكية كلية الطب البيطرى جامعه المنصوره وكفر الشيخ

قسم علم الحيوان كلية العلوم جامعه بنها

ان الطفيليات المعوية في الحيوانات المنتجة لها قيمة اقتصادية عالية ، وبالتالي فإن العلاج يساعد على الحفاظ على أرباح عالية. وبما أن ليس بالكثير معروف عن استخدام الأدوية لعلاج اصابات الجاموس، فقد كان الهدف من هذه الدراسة هو وصف النتائج السريرية ونتائج علاج داء التوكسوكاريا في الجاموس في منطقة الدلتا بمصر. أجريت الدراسة على عدد مائتى وأربعة جاموسة مختلفة العمر والجنس للكشف عن وجود توكسوكارا فيتولوروم على مدى ستة أشهر في منطقة الدلتا، مصر. وتم تحديد توكسوكارا فيتولوروم في ١٦.٦٪ من الجاموس التي تم فحصها حيث كان المرض أكثر انتشارا في العجول الأقل من ستة أشهر. وتراوحت ردود الفعل السريرية من لا شيء إلى شديد وفقا لعمر الحيوان وشدة اصابته. وكانت علامات الاصابة هي عدم الحضور، والإسهال أو الإمساك، الطبل الطفيف والوحم في معظم الحالات. بينما لم تتغير درجة حرارة الجسم في جميع الحالات المصابة. وفيما يتعلق بنتائج فحص الدم، فلقد قلت مستويات الهيموجلوبين، وحجم الخلايا المكدسة، وإجمالي عدد كريات الدم الحمراء في العجول المصابة مقارنة بالشواهد. بينما لوحظ كثرة الحمضات في العجول المصابة مقارنة بالشواهد. وعندما تم تقييم عقار الإيفرميكتين، والبييرازين سترات في هذه الحيوانات المصابة طبيعيا، أدى استخدام هذين العقارين الطاردي للديدان الى التخلص من الديدان وتطبيع المعالم السريرية والدمية بعد أسبوعين وأربعة أسابيع ، على التوالي.

١٣. المجلة المصرية للعلوم الطبية ٣٧(٢) ديسمبر ٢٠١٦ : ٧١٩-٧٢٦.