Global Veterinaria 14 (5): 646-655, 2015
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2015.14.05.94137

Control of Cryptosporidiosis in Buffalo Calves Using Garlic (*Allium sativum*) and Nitazoxanide with Special Reference to Some Biochemical Parameters

[1]Kadria N. Abdel Megeed, [2]A.M. Hammam, [3]G.H. Morsy,
[1]Fathia A.M. Khalil, [2]M.M.E. Seliem and [1]Dina Aboelsoued

[1]Parasitology and Animal Diseases Department, National Research Centre, Dokki, Giza, Egypt
[2]Animal Reproduction and A.I. Department, National Research Centre, Dokki, Giza, Egypt
[3]Zoology Department, Faculty of Science, Benha University, Benha, Egypt

Abstract: Clinical examination of 100 diarrheic buffalo calves from different farms at Qualiobya province was performed. Diarrhea, apathy, lack of appetite, mild fever and dehydration were the major signs observed. For the goal of treating Cryptosporidiosis, Two treatment trials were conducted in this study on 36 naturally *Cryptosporidium*-infected buffalo calves using garlic and Nitazoxanide (NTZ). There was a gradual reduction in oocyst counts in garlic and NTZ treated animals; this reduction was more obvious in garlic than NTZ treated groups and continued till reaching negligible degree or no oocysts detection at 15 to18 days post treatment in garlic and at 3 weeks in NTZ treated animal groups. Then, after one week, the majority of NTZ treated calves excreted oocysts at low level. Oocysts observed during treatments were found to be deformed in shape. Dealing with biochemical changes, there was a significant increase in RBCs and WBCs counts, Hemoglobin concentrations, PCV and potassium levels and a significant decrease in total protein, albumin and globulin concentrations, A/G ratio, glucose, sodium, chloride, iron and copper concentrations in the infected animals than the healthy ones. Almost all biochemical parameters tested enhanced in their concentrations towards the healthy normal status after treatments. Garlic offered a promising effect in the treatment of cryptosporidiosis.

Key words: Buffalo Calves · *Cryptosporidium* · Diarrhea · Garlic · Nitazoxanide · Biochemical Parameters

INTRODUCTION

Cryptosporidium is a protozoan parasite infecting bovines, goats, pigs, dogs and cats and considered as an important co-factor in neonatal diarrhea in cattle, sheep, goats and water buffaloes [1,2]. Concerning buffaloes, Egypt had about 4 million heads or ~2.3% of the world water buffalo population. Buffaloes is considered a main asset of the Egyptian livestock industry [3, 4].

Previous studies had indicated that rotaviruses, coronaviruses, *E. coli* and *C. parvum* were all contributing factors to diarrhea in calves. However, *C. parvum* was believed to be the primary cause of calf diarrhea [10]. Cryptosporidiosis was characterized by acute gastro-intestinal disturbances, mucoid to hemorrhagic diarrhea, low grade fever and loss of condition but infection could be asymptomatic [11, 12]. *Cryptosporidium* was increasingly recognized as one of the major cause of moderate to severe diarrhea [13, 14]. *Cryptosporidium* infected buffalo calves showed yellowish, greenish or clay colored profuse watery diarrhea, anorexia, colicy pain, normal body temperature, reduced milk suckling and with progression of the disease, animals dehydrated with evidence of lying down posture [6, 7]. Chronic cryptosporidiosis could develop in high risk host groups, particularly neonates [15]. The risk of developing severe disease differed depending on the host immune status [16].

Control of cryptosporidiosis remained a global challenge in both veterinary and human medicine. Thousands of agents had been tested both *in vivo* and *in vitro.* Some were active *in vitro* but exhibited poor or

Corresponding Author: Dina Aboelsoued, Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Centre, Post Box: 12622, El-Tahrir Street, Dokki, Cairo, Egypt.
Tel: +01000406772, E-mail: dr.dina.aboelsoued@gmail.com.

no response in clinical trials [17]. Some reports tested the effect of plant extracts in the treatment of cryptosporidiosis such as: garlic [18, 19], pine-bark extract [20], blueberry extracts [21], curcumin [22], onion and cinnamon [23] and black seed [24]. Garlic (*Allium sativum*) was a convenient prophylactic and a promising therapeutic agent for cryptosporidial infection. The efficacy of garlic in the prophylaxis and treatment of experimental cryptosporidiosis could be explained by different mechanisms [25]. El Shenaway *et al.* [26] reported the enhancement of phagocytosis and an increase in natural killer cell activity which promoted the immune system function and strengthened the body's defense mechanism during the duration of treatment by garlic. Furthermore, Masamha *et al.* [27] found that *Allium sativum* disrupted the normal physiological functions of the parasite like mobility, food absorption and reproduction. Also, many therapeutic substances had been tested for the prophylaxis of cryptosporidiosis in ruminants such as: Paromomycin [28], α, β-cyclodextrin [29], Mangiferin [30], Neopredisan [31], NTZ [32], Azithromycin and Co-trimoxazole [24] and Halofuginone lactate [33]. Despite exhaustive attempts at chemotherapy, no effective treatment for cryptosporidiosis had been identified [34]. However, NTZ might be promising [35]. It significantly shortened the duration of diarrhea and decreased mortality in adults and in malnourished individuals [36].Diarrhea was resolved by NTZ within 3 or 4 days of initiation. NTZ treatment reduced the duration of both diarrhea and oocyst shedding [37].

MATERIALS AND METHODS

Animals: Clinical examination of 100 diarrheic buffalo calves from different farms at Qualiobya province was carried out according to Rosenberger *et al.* [38]. Those animals were classified into 3 age groups; 40 buffalo calves aged less than month, 30 buffalo calves aged 1-2 months and 30 buffalo calves aged 2 months.

Treatment experiments were conducted on 36 naturally *Cryptosporidium* infected buffalo calves and 12 control healthy buffalo calves in the farm of Animal Reproduction Research Institute, Giza, Egypt.

Cryptosporidium-infected animal groups were classified as follows; Group A: 12 control positive untreated buffalo calves, Group B: 12 buffalo calves received garlic preparation as a plant treatment and Group C: 12 buffalo calves received Nitazoxanide as a medical treatment.

Each group was further divided into 3 age groups (4 animals for each); Buffalo calves aged less than one month, from 1-2 months and 2 months.

- *Allium Sativum (Garlic) Preparation:* Garlic was administrated to the experimental animals as crude juice. The crude extract was prepared according to Masamha *et al.* [27]. Animals received garlic orally as a crude juice in a dose of 3.5 g once daily for 2 weeks. The dose selected for the present work was 50 mg/kg body weight [39].
- *Nitazoxanide Treatment:* Animals received Cryptonaz® (Copad Pharma, Egypt) orally in a dose 25ml (500mg Nitazoxanide) twice daily, every 12 hours, for 3 days.

Fecal samples were collected from treated and untreated animal groups every 3 days after treatments for six weeks, smeared on a slide and stained with MZN technique. The number of oocysts output was counted for each group in 50 fields (Oil immersion) of MZN stained fecal smears [40].

Detection of oocysts: Fine feces smears fixed with methanol spirit and stained with Modified Ziehl-Neelsen Stain (MZN) for detection of *Cryptosporidium* oocysts [41]. The preparations were examined and the oocysts were measured with help of stage micrometer conjugated with the light microscope at the eyepiece 10x and the objective 100x. All measurements are in micrometers (µm) for about 20-50 oocysts, with the range in parenthesis following the mean [42].

Blood samples were collected from healthy and infected animal groups at zero time and 10 days after treatments. Blood samples were collected from each animal into two tubes; the first was heparinized and used for determination of hematological parameters. While, the second portion was placed in a plain centrifuge tube for serum separation and used for biochemical analysis.

Hematological Tests: RBCs and WBCs counts, PCV and Hb were read by Hematology Analyzer (Perlong Medical Machine Co., Ltd., China).

Biochemical Analysis:

- The concentrations of serum total protein (Biuret Method) and albumin were determined using reagent kits obtained from Biodiagnostic Co., Giza, Egypt [43 and 44]. Serum globulin concentration was calculated

by subtracting the obtained value of albumin from the total proteins. Also, Albumin/Globulin (A/G) ratio was obtained by dividing value of albumin on globulin.

- Blood glucose and serum Iron and Copper levels were estimated by colorimetric methods using reagent kits obtained from Biodiagnostic Co., Giza, Egypt [45-47].
- Serum sodium, Potassium and Chloride levels were estimated using atomic absorption spectrophotometer (Perkin-Elmer-1100B, USA).

Statistical Analysis: Data were analyzed for the means and standard deviations. Significance of the results was evaluated using Analysis of variance (ANOVA) and Duncan using Statistical Package for Social Science (SPSS) computer programs [48].

RESULTS

The Clinical Signs Observed in Diarrheic Calves: Greenish yellow mucoid diarrhea, often bloody, apathy, lack of appetite, mild fever and dehydration were the major signs observed. Symptoms were highly detected in young buffalo calves aging less than one month and decreased with increasing age (Table 1).

Counts and Shape of *Cryptosporidium* Oocysts Before and after Treatment with Garlic and NTZ: Examination of fecal smears for detection of *Cryptosporidium* oocyst counts revealed that there was a gradual reduction in oocysts shedding in garlic treated animals than infected non-treated ones in all age groups and continued till reaching negligible numbers or no oocysts detection at 15 to 18 days post treatment in garlic treated groups (Table 2). There was reduction in oocysts shedding at all NTZ treated animal groups than infected non-treated groups and continued till reaching negligible numbers or no oocysts were detected at 3 weeks post treatment. Then, after one week, the majority of NTZ treated calves returned to excrete oocysts at low level (Table 3). The reduction in oocysts shedding in the treated groups was more obvious in garlic than NTZ treated groups. Animal age had an effect on the reaction of the animal towards the treatment making older animals more susceptible to be cured faster. In the *Cryptosporidium* infected non-treated groups, the oocysts were best visualized by MZN stain as spherical to ovoid pink organisms, 5.1 x 4.6µm in diameter. Oocysts observed post garlic and NTZ treatments were found to be deformed in shape lacking any inner structures (Sporozoites) (Fig. 1).

Biochemical Parameters: It could be seen from (Table 4) that RBCs counts, WBCs counts, PCV percentage and hemoglobin concentrations showed a significant *(P<0.001)* increase in *Cryptosporidium* infected animals than the apparently healthy ones.

There was a significant decrease (*P<0.001*) in the total protein, albumin and globulin concentrations in the control infected animals than the apparently healthy animals (Table 5).

It was clear from (Table 6) that there was a significant (*P<0.001*) decrease in glucose concentrations in the control infected animals than the apparently healthy ones. There was a significant (*P<0.001*) decrease in sodium, chloride, iron and copper concentrations in the control infected animals than the apparently healthy animals. Potassium concentration increased significantly (*P<0.001*) in the control infected animals than the apparently healthy animals (Table 6).

Table 1: The clinical signs observed in diarrheic calves.

		Age Groups					
		Less than 1 month(N = 40 animals)		1-2 months(N = 30 animals)		2 months(N= 30 animals)	
Signs		Number	Percent	Number	Percent	Number	Percent
Yellow mucoid Diarrhea	With blood	26	65%	10	33.3%	8	26.7%
	Without blood	14	35%	20	66.7%	22	73.3%
Mild fever(40-41°C)		15	37.5%	9	30%	6	20%
Dehydration		31	77.5%	19	63.3%	12	40%
Anorexia		36	90%	22	73.3%	17	56.7%
Apathy		30	75%	18	60%	9	30%
Posture (Calves lying down)		35	87.5%	21	70%	13	43.3%

Table 2: Counts of *Cryptosporidium* oocysts in infected untreated and infected treated buffalo calves with garlic every three days.

	Groups						
	Infected untreated Animals			Garlic Treated Animals			
Days post treatment	Age less than 1 month	Age1-2 months	Age2 months	Age less than 1 month	Age1-2 months	Age2 months	F-Value
Day 18	67±3.6^{bc}	78±5.7^{a}	73.8±9.7^{ab}	64.3±5.1^{c}	61.5±5.1^{c}	52±2.2^{d}	10.4**
Day 21	65.3±1.3^{ab}	71.8±10.1^{a}	72.8±12^{a}	58.8±8.3^{b}	43±2.2^{c}	31±2.5^{d}	20.2**
Day 24	63.5±2.7^{b}	76.5±3.8^{a}	68±10.3^{ab}	24±8.5^{c}	23±4.1^{c}	22.5±4.8^{c}	66**
Day 27	58±7.3^{a}	57.5±16.9^{a}	45.8±5.3^{a}	18.8±5.9^{b}	20±4.1^{b}	19.5±2.6^{b}	21.1**
Day 30	24±8.5^{a}	23±4.1^{a}	22.5±4.8^{a}	15±4.2^{b}	13.3±4.4^{b}	10.3±1^{b}	5.5*
Day 33	18.75±6^{a}	20±4.1^{a}	19.5±2.6^{a}	8±1.6^{b}	6.3±4.5^{bc}	2±2.4^{c}	17.1**
Day 36	8.5±0.6^{a}	6.5±0.6^{ab}	6±0.01^{ab}	6±1.4^{ab}	4.8±1.3^{b}	1.3±2.5^{c}	4.5*
Day 39	2±1.1	2.2±1.1	2.5±1	4.5±1.3	4.5±2.9	1±0.9	1.5$^{N.S.}$
Day 42	1±0.9	1±0.9	1±1.4	3.7±1.5	4±2.2	1±0.9	2.5$^{N.S.}$
Day 45	5.3±0.5^{a}	3.8±0.5^{a}	3.8±0.5^{a}	3.8±1.5^{a}	4±1.1^{a}	0.8±0.5^{b}	3.5*
Day 48	4±2.9	1.8±0.4	0.5±0.3	0.5±0.3	0.5±0.2	0.00	2.2$^{N.S.}$
Day 51	1.8±1.3	1.8±1.3	1.3±1	0.5±0.2	0.5±0.4	0.00	0.8$^{N.S.}$
Day 54	5.8±0.9^{a}	5.3±1.3^{a}	4.3±3.1^{a}	0.5±0.01^{b}	0.3±0.01^{b}	0.00^{b}	13.1**
Day 57	8±2.9^{a}	4±3.9^{bc}	5.5±3.1^{ab}	0.5±0^{cd}	0.00^{d}	0.00^{d}	7.8**
Day 60	6.3±3.5^{a}	1.8±2.4^{b}	0.5±0.4^{b}	0.00^{b}	0.00^{b}	0.00^{b}	7.7**

All data expressed as Mean ± SD.

* Significant differences at $P < 0.05$, ** Significant differences at $P < 0.001$.

N.S. Non-significant, Means followed by different letters indicated significance.

Table 3: Counts of *Cryptosporidium* oocysts in infected untreated and infected treated buffalo calves with NTZ every three days.

	Groups						
	Infected untreated Animals			NTZ Treated Animals			
Days post treatment	Age less than 1 month	Age1-2 months	Age2 months	Less Than 1 month	Age1-2 months	Age2 months	F-Value
Day 6	113.3±4.7^{a}	98±3.2^{b}	87±10.2^{bc}	110.3±2.9^{a}	91±7.6^{bc}	83.3±11.3^{c}	11.1**
Day 9	110.3±2.9^{a}	90.5±6.7^{b}	82±12.7^{bc}	66.3±2.2^{d}	80.5±6.7^{bc}	71.3±8.7^{cd}	17.3**
Day 12	110±2.8^{a}	91±7.6^{b}	83.3±11.3^{b}	65.3±1.3^{c}	71.8±8.6^{c}	62.5±8.7^{c}	22.6**
Day 15	107.8±2.5^{a}	90.5±8.2^{b}	82±13.1^{b}	43.3±8.3^{c}	54.3±5.4^{c}	47.3±5^{c}	45.2**
Day 18	67±3.6^{b}	78±5.8^{a}	73.8±9.7^{ab}	18.8±6^{c}	20±4.1^{c}	19.5±2.6^{c}	104.8**
Day 21	65.3±1.3^{a}	71.8±10.1^{a}	72.8±12.4^{a}	6±2.5^{b}	4.8±2.2^{b}	4±1.2^{b}	108.6**
Day 24	63.5±2.6^{b}	76.5±3.8^{a}	68±10.3^{b}	1.8±2.4^{c}	1.8±2.3^{c}	0.5±0.^{c}	241.7**
Day 27	58±7.3^{a}	57.5±16.8^{a}	45.8±5.3^{b}	0.5±0.1^{c}	0.00^{c}	0.00^{c}	57.7**
Day 30	24±8.5^{a}	23±4.1^{a}	22.5±4.8^{a}	0.00^{b}	0.00^{b}	0.00^{b}	34.7**
Day 33	18.8±6^{a}	20±4.1^{a}	19.5±2.6^{a}	0.00^{b}	0.00^{b}	0.00^{b}	45.9**
Day 36	8.5±0.6	6.5±0.6	6±0.01	7.8±5.3	2.8±1.7	6.3±2.5	1.6$^{N.S.}$
Day 39	2±0.4	2±0.5	2.5±1.01	3.8±1.5	4±1.16	3±0.01	0.7$^{N.S.}$
Day 42	1±0.2	1±0.2	1±0.3	1.8±0.4	1.8±0.4	0.5±0.1	0.35$^{N.S.}$
Day 45	5.3±0.5^{a}	3.8±0.5^{b}	3.8±0.5^{b}	0.00^{c}	0.5±0.1^{c}	1±0.2^{c}	35.9*
Day 48	4±1.3	1.8±0.3	0.5±0.1	7.8±5.3	2.8±1.7	5.3±1.5	1.9$^{N.S.}$
Day 51	1.8±0.3	1.8±0.3	1.3±0.5	7±1.8	3.5±1.3	5.5±2.1	2.01$^{N.S.}$
Day 54	5.8±1	5.3±1.2	4.3±1.1	7.8±1.5	8±2	6.8±1.7	2.44$^{N.S.}$
Day 57	8±2.4	4±1.3	5.5±2.1	5.8±1	5.5±1	4±1.2	1.14$^{N.S.}$
Day 60	6.3±2.5^{a}	1.8±0.4^{bc}	0.5±0.1^{c}	5.8±1.3^{a}	5.3±1.3^{ab}	4.3±1^{ab}	4.2*

All data expressed as Mean ± SD.

* Significant differences at $P < 0.05$, ** Significant differences at $P < 0.001$.

N.S. Non-significant, Means followed by different letters indicated significance.

NTZ: Nitazoxanide treatment.

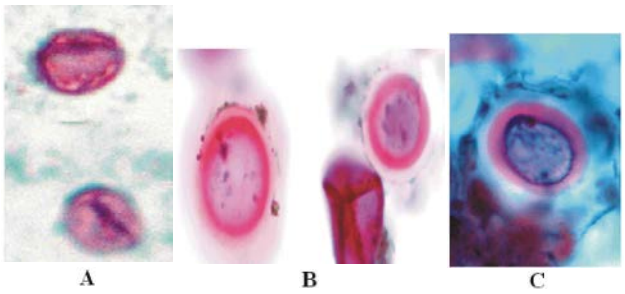

Fig. 1: *Cryptosporidium* oocysts in stained fecal smears of infected non-treated (A), garlic-treated (B) and NTZ-treated (C) buffalo calves (MZN X1000)

Table 4: Mean values of some hematological parameters in apparently healthy and naturally *Cryptosporidium* infected buffalo calves before and after treatments

	Item				
		Cryptosporidium infected buffalo calves			
			Post treatment		
Parameter	Apparently Healthy Animals	Control Infected Animals	"Garlic"	"NTZ"	F-Value
RBCs count (number×10^{12}/L)	7.74 ± 0.28 [b]	8.16 ± 0.16 [a]	7.92 ± 0.12 [b]	7.87 ± 0.09 [b]	7.7**
Hb (g/dl)	10.47 ± 0.13 [c]	11.2 ± 0.11 [a]	10.88 ± 0.11 [b]	10.94 ± 0.07 [b]	64.3**
PCV (%)	35.18 ± 0.45 [c]	38.53 ± 0.42 [a]	36.99 ± 0.06 [b]	37.02 ± 0.08 [b]	156**
WBCs count (number×10^{9}/L)	8.41 ± 0.07 [c]	9.52 ± 0.06 [a]	8.74 ± 0.05 [b]	8.74 ± 0.04 [b]	586.6**

All data expressed as Mean ± SD., **Significant differences at $P < 0.001$.
Means followed by different letters indicated significance.

Table 5: Mean values of serum protein levels in healthy and naturally *Cryptosporidium* infected buffalo calves before and after treatment.

	Item				
		Cryptosporidium infected buffalo calves			
			Post treatment		
Parameter	Apparently Healthy Animals	Control Infected Animals	"Garlic"	"NTZ"	F-Value
Total Protein (g/dl)	7.64 ± 0.03 [a]	6.05 ± 0.04 [d]	7.12 ± 0.13 [b]	7.04 ± 0.05 [c]	647.86**
Albumin (g/dl)	3.54 ± 0.07 [a]	2.48 ± 0.08 [c]	3.08 ± 0.05 [b]	3.11 ± 0.07 [b]	309.93**
Globulin (g/dl)	4.1 ± 0.06 [a]	3.57 ± 0.08 [c]	4.04 ± 0.14 [a]	3.92 ± 0.05 [b]	55.5 **
A/G Ratio	0.86 ± 0.03 [a]	0.7 ± 0.04 [c]	0.76 ± 0.04 [b]	0.79 ± 0.03 [b]	35.03 **

All data expressed as Mean ± SD., **Significant differences at $P < 0.001$.
Means followed by different letters indicated significance.

Table 6: Mean values of some serum biochemical parameters in healthy and naturally *Cryptosporidium* infected buffalo calves before and after treatment.

	Item				
		Cryptosporidium infected buffalo calves			
			Post treatment		
Parameter	Apparently Healthy Animals	Control Infected Animals	"Garlic"	"NTZ"	F-Value
Glucose (mg/dl)	72.4 ± 0.41 [a]	61.8 ± 0.56 [c]	70.55 ± 0.46 [b]	70.72 ± 0.25 [b]	975.2 **
Sodium (m.Eq/L)	132.11 ± 0.85 [a]	123 ± 1.34 [c]	129.48 ± 0.54 [b]	128.9 ± 0.39 [b]	159.18**
Potassium (m.Eq/L)	4.40 ± 0.04 [c]	5.84 ± 0.05 [a]	4.47 ± 0.04 [b]	4.47 ± 0.03 [b]	2674.2**
Chloride (m.Eq/L)	96.31 ± 0.2 [a]	94.23 ± 0.1 [c]	95.33 ± 0.21 [b]	95.39 ± 0.04 [b]	236.47**
Iron (µg %)	254.28 ± 0.2 [a]	230.13 ± 0.08 [c]	250 ± 0.46 [b]	250.03 ± 0.44 [b]	8450.4**
Copper (µg%)	85.39 ± 0.3 [a]	71.2 ± 0.8 [c]	81.36 ± 0.86 [b]	81.66 ± 0.69 [b]	612.68**

All data expressed as Mean ± SD., **Significant differences at $P < 0.001$.
Means followed by different letters indicated significance

DISCUSSION

In this study, symptoms were highly detected in young buffalo calves aged less than 1 month and decreased with age. Similar observations were reported by Göz *et al*. [49] and Osman and Sadiek [50]. Also, Lassen *et al.* [51] found that diarrhea was only associated with *Cryptosporidium* in young calves aged less than 3 months.

The search for bioactive plants which could be used as anti-parasitic treatment had received considerable attention in recent years because of the increasing worldwide development of resistance to chemical drugs in parasitic populations. In this study, the allicin was obtained from crushed fresh garlic bulbs [27, 52] and the selected dose was 50 mg/kg body weight supposing that this dose was equivalent to the daily amount of garlic recommended for an average human to maintain good health (~4 g) [39]. In this study, garlic successfully eradicated the *Cryptosporidium* oocysts from the feces and almost no oocysts were detected in the feces of garlic treated groups by the end of the experiment. This impressive effect of garlic was similarly obtained using garlic bulbs and garlic extract [25, 18].

Drug treatment caused a marked decrease in the mean number of oocysts after the initiation of therapy in NTZ treated animal groups. The results showed no significant difference in most days recorded between the infected non treated animal groups and NTZ treated animals groups at all age groups. In the NTZ treated animal groups and the untreated animal groups, mean numbers of oocysts continued their reduction daily till they were almost near zero. It was clear that animals produced a self-limited action towards the disease in the untreated groups. Similar findings had been recorded in several studies including *in vitro* and *in vivo* studies using several animal models and clinical trials, which had demonstrated the effectiveness of NTZ in treating diarrhea and enteritis caused by *Cryptosporidium* species [16, 53]. After 3 weeks, no oocysts were detected in the feces of NTZ treated animals at all ages. Then, after one week, the majority of NTZ treated calves excreted oocysts at low level. This so-called "Rebound phenomenon" had been reported by previous workers [54, 55]. Explanations for its occurrence might include re-infection, activation of an auto-infection phase with thin-wall oocysts and/or re-activation of inhibited stages [56]. Another possibility was that the older calves might have been infected with different *Cryptosporidium* species [55]. Using NTZ in *Cryptosporidium*-infected calves did not show the expected positive effect on the course of infection, neither on reducing the clinical severity, nor on oocysts excretion [32]. The present results suggested that garlic and NTZ had a great effect on *Cryptosporidium* oocyst counts and animals were almost negative to *Cryptosporidium* infection after treatments. However, garlic gave better results as it was suggested to raise the animals' immunity not to excrete more oocysts after reaching zero.

The present study showed that there was a significant increase in RBCs count and Hb concentration in the infected calves than apparently healthy ones. These findings agreed with Osman and Sadiek [50] and differed from El-Dessouky and El-Masry [7]. There was a non-significant difference between treated groups than the apparently healthy group. These non-significant changes in the mean values of RBCs and Hb concentrations in treated calves than the apparently healthy ones agreed with those reported by some authors [7, 50, 57]. The expected reduction of RBCs counts and Hb concentrations due to anorexia and diarrhea could be masked by the expected increase in their values might be due to longstanding diarrhea and dehydration and the subsequent hemoconcentration [58]. Mean values of PCV showed highly significant increase in infected group. These results agreed with Osman and Sadiek [50], reflecting excessive loss of body fluid in diarrheic calves, in addition to inadequate intake of milk and fluids during diarrhea [59]. The significant increase in WBCs counts in infected buffalo calves which agreed with some authors [7 and 50] could be declared according to Malina *et al*. [60] stating that the infection with cryptospordia or any protozoa like parasites lead to increase in the WBCs or it might reflect the associating bacterial infection.

Regarding the mean values of serum total protein, albumin and globulin levels, they were significantly decreased in *Cryptosporidium* infected buffalo calves in comparison to the apparently healthy ones. These results were supported by similar findings recorded by some authors [7, 57, 61]. This reduction in serum total protein and albumin in diseased calves could be attributed to poor absorption of nutrients and excessive protein breakdown and loss of albumin [60]. Decreased globulins might be due to marked depression in gamma globulins fraction associating with the diseased condition [62].

The high significant decrease in the blood glucose concentrations in infected buffalo calves than apparently healthy ones agreed with those obtained by El-Dessouky and El-Masry [7] and Osman and Sadiek [50]. These results could be related to lack of glucose

absorption from damaged intestine where the *Cryptosporidium* caused damage of villi and impaired digestion and absorption [63].

The significant decrease in sodium and chloride levels in infected buffalo calves than apparently healthy ones agreed with El-Dessouky and El-Masry [7] and Osman and Sadiek [50]. These results could be attributed to the loss of large amounts of sodium and chloride ions with intestinal secretion and diarrhea [59]. There was a highly significant increase in potassium (K) levels in infected animals than healthy ones. These findings were confirmed by the results of Tawfik *et al.* [64] and could be attributed to the subsequent acidosis associating longstanding enteritis in which false positive increase in K values in attempt to compensate the occurring metabolic acidosis which known as K/H exchange [65]. This obvious increase in serum potassium levels might be related to the increased osmotic pressure in the lumen of the gut owing to an increase in the number of smaller molecules such as volatile fatty acids [66]. Hyperkalemia was a common problem associated with the acid-base and electrolyte disturbances that occur in neonate diarrheic calves [67].

Iron and copper levels decreased significantly in infected buffalo calves than the apparently healthy ones. After treatments, there was a non-significant difference between treated groups and apparently healthy group. These results partially agreed with El-Dessouky and El-Masry [7] who found a non-significant difference in copper levels at healthy, infected and treated groups. This might be attributed to the decrease in beta globulin levels which had specific mineral binding capacity or to the anorexic condition of the diarrheic calves [68].

Almost all measured biochemical parameters after garlic and NTZ treatments enhanced in their concentrations towards the healthy normal status. This means that garlic and NTZ treatments were effective not only in reducing oocyst counts, but also in making the animals health better. The absence of significant differences between the two treated groups in the parameters' levels recorded assuming that both treatments had almost the same effect on biochemical status of the buffalo calves.

In conclusion, *Cryptosporidium* was a parasite which had the ability to induce diarrhea in buffalo calves. *Cryptosporidium* is considered a problem of neonate calves. Garlic offered a promising effect in the treatment of cryptosporidiosis and it's recommended to be used in the daily animal diet to give protective effects against parasites.

REFERENCES

1. Fayer, R., M. Santín and L. Xiao, 2005. *Cryptosporidium bovis n. sp*. (Apicomplexa: Cryptosporidiidae) in cattle (*Bos taurus*). J. Parasitol., 91: 624-629.
2. Fayer, R., M. Santín and J.M. Trout, 2008. *Cryptosporidium ryanae n. sp*. (Apicomplexa: Cryptosporidiidae) in cattle (*Bos taurus*). Vet. Parasitol., 156: 191-198.
3. Wilson, R.T., 2012. The past and present of and potential for the domestic (water) buffalo in Africa. Trop. Anim. Health Prod., 44: 1367-1373.
4. Amer, S., S. Zidan, Y. Feng, H. Adamu, N. Li and L. Xiao, 2013. Identity and public health potential of *Cryptosporidium spp*. in water buffalo calves in Egypt. Vet. Parasitol., 191: 123-127.
5. Fathia, A.M. Khalil, 1993. Studies on Cryptosporidium infection in calves M.V.Sc. Thesis, Fac. Vet. Med., Cairo University, Egypt.
6. El-Sherif, A.M., M.A. Abdel-Gawad, H.S. Lotfy and K.A.M. Shokier, 2000. Impact of gastrointestinal nematodes and some enteric protozoalaffections on the health of buffalo calves. Assiut. Vet. Med. J., 43: 260-270.
7. El-Dessouky, S.A. and N.M. El-Masry, 2005. Effect of *Cryptosporidium parvum* infection on the haematology and blood chemistry of buffalo calves with special reference to the prevalence of infection in adult buffaloes. Assiut Vet. Med. J., 51: 108-123.
8. El-Khodery, S.A. and S.A. Osman, 2008. Cryptosporidiosis in buffalo calves (*Bubalus bubalis*): Prevalence and potential risk factors. Trop. Anim. Health Prod., 40: 419-426.
9. Shoukry, N.M., H.A. Dawoud and F.M. Haridy, 2009. Studies on zoonotic cryptosporidiosis parvum in Ismailia Governorate, Egypt.J. Egypt. Soc. Parasitol., 39: 479-488.
10. Chalmers, R.M., R. Smith, K. Elwin, F.A. Clifton-Hadley and M. Giles, 2011. Epidemiology of anthroponotic and zoonotic human cryptosporidiosis in England and Wales, 2004–2006. Epidemiol. Infect., 139: 700-712.
11. Klein, P., T. Kleinovα, Z. Volek and J. Šimůne, 2008. Effect of *Cryptosporidium parvum* infection on the absorptive capacity and paracellular permeability of the small intestine in neonatal calves. Vet. Parasitol., 152: 53-59.

12. Cui, Z., R. Wang, J. Huang, H. Wang, J. Zhao, N. Luo, J. Li, Z. Zhang and L. Zhang, 2014. Cryptosporidiosis caused by *Cryptosporidium parvum* subtype IIdA15G1 at a dairy farm in Northwestern China. Parasit. Vectors, 7: 529-532.
13. Ryan, U., R. Fayer and L. Xiao, 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitol., 141(13): 1667-1685.
14. Lendner, M., D. Böttcher, C. Delling, K.K. Ojo, W.C. Van Voorhis and A. Daugschies, 2015. A novel CDPK1 inhibitor-a potential treatment for cryptosporidiosis in calves? Parasitol. Res., 114: 335-336.
15. Keating, J.P., 2005. Chronic diarrhea. Pediatr. Rev., 26: 5-14.
16. Abdou, A.G., N.M. Harba, A.F. Afifi and N.F. Elnaidany, 2013. Assessment of *Cryptosporidium parvum* infection in immunocompetent and immunocompromised mice and its role in triggering intestinal dysplasia. Int. J. Infect. Dis., 17: 593-600.
17. Shahiduzzaman, M. and A. Daugschies, 2012. Therapy and prevention of cryptosporidiosis in animals. Vet. Parasitol., 188: 203-214.
18. Wahba, A.A., 2003. Studies on the efficacy of garlic extract on Cryptosporidiosis in experimentally infected mice. Egypt. J. Agricul. Res., 81: 795-805.
19. Toulah, F.H., A.A. El-Shafei and H.S. Al-Rashidi, 2012. Evaluation of garlic plant and indinavir drug efficacy in the treatment of cryptosporidiosis in experimentally immumosuppressed rats. J. Egypt. Soc. Parasitol., 42: 321-328.
20. Kim, H.C. and J.M. Healey, 2001. Effects of pine bark extract administered to immunosuppressed adult mice infected with *Cryptosporidium parvum*. Am. J. Chin. Med., 29: 469-475.
21. Anthony, J.P., L. Fyfe, D., Stewart, G.J. McDougall and H.V. Smith, 2007. The effect of blueberry extracts on *Giardia duodenalis* viability and spontaneous excystation of *Cryptosporidium parvum* oocysts, *in vitro*. Methods, 42: 339-348.
22. Shahiduzzaman, M., V. Dyachenko, R.E. Khalafalla, A.Y., Desouky and A. Daugschies, 2009. Effects of curcumin on *Cryptosporidium parvum in vitro*. Parasitol. Res., 105: 1155-1161.
23. Abu El Ezz, N.M.T., A.M. Khalil and R.M. Shaapan, 2011. Therapeutic Effect of Onion (*Allium cepa*) and Cinnamon (*Cinnamomum zeylanicum*) Oils on Cryptosporidiosis in Experimentally Infected Mice. Global Vet., 7: 179-183.
24. Nasir, A., M.Avais, M.S. Khan, J.A. Khan, S. Hameed and M.P. Reichel, 2013. Treating *Cryptosporidium parvum* infection in calves. J. Parasitol., 99: 715-717.
25. Gaafar, M.R., 2012. Efficacy of *Allium sativum* (Garlic) against experimental Cryptosporidiosis. Alex. J. Med., 48: 59-66.
26. El Shenawy, N.S., M.F. Soliman and S.I. Reyad, 2008. The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as antischistosomiasis agents in mice. Rev. Inst. Med. Trop. Sao Paulo, 50: 29-36.
27. Masamha, B., C.T. Gadzirayi and I. Mukutirwa, 2010. Efficacy of *Allium sativum* (Garlic) in controlling nematode parasites in sheep. Inter. J. Appl. Res. Vet. Med., 8: 161-169.
28. Grinberg, A., A. Markovics, J. Galindez, N. Lopez-Villalobos, A. Kosak and V.M. Tranquillo, 2002. Controlling the onset of natural cryptosporidiosis in calves with *paromomycin sulphate*. Vet. Rec., 151: 606-608.
29. Castro-Hermida, J.A., I. Pors, F. Otero-Espinar, E. Luzardo-Alvarez, E. Ares-Mazαs and C. Chartier, 2004. Efficacy of α, β-cyclodextrin against experimental cryptosporidiosis in neonatal goats. Vet. Parasitol., 120: 35-41.
30. Perrucci, S., G. Fichi, C. Buggiani, G. Rossi and G. Flamini, 2006. Efficacy of mangiferin against *Cryptosporidium parvum* in a neonatal mouse model. Parasitol. Res., 99: 184-188.
31. Najdrowski, M., A. Joachim and A. Daugschies, 2007. An improved *in vitro* infection model for viability testing of *Cryptosporidium parvum* oocysts. Vet. Parasitol., 150: 150-154.
32. Schnyder, M., L. Kohler, A. Hemphill and P. Deplazes, 2009. Prophylactic and therapeutic efficacy of Nitazoxanide against *Cryptosporidium parvum* in experimentally challenged neonatal calves. Vet. Parasitol., 160: 146-154.
33. Trotz-Williams, L.A., B.D. Jarvie, A.S. Peregrine, T. Duffield and K.E. Leslie, 2011. Efficacy of halofuginone lactate in the prevention of cryptosporidiosis in dairy calves. Vet. Rec., 168: 509.
34. Mead, J.R., 2002. Cryptosporidiosis and the challenges of chemotherapy. Drug Resist Updat., 5: 47-57.
35. Ollivett, T., D. Nydam, D. Bowman, J. Zambriski, M. Bellosa, T. Linden and T. Divers, 2009. Effect of Nitazoxanide on cryptosporidiosis in experimentally infected neonatal dairy calves. J. Dairy Sci., 92: 1643-1648.

36. Gargala, G., 2008. Drug treatment and novel drug target against *Cryptosporidium*. Parasite, 15: 275-281.
37. Rossignol, J.F., A. Ayoub and M.S. Ayers, 2001. Treatment of diarrhea caused by *Cryptosporidium parvum*: a prospective randomized, double-blind, placebo-controlled study of nitazoxanide. J. Infect. Dis., 184: 103-106.
38. Rosenberger, G., G. Dirksen, H.D. Grunder, E. Grunert, D. Krause and M. Stober, 1979. Clinical examination of cattle. Eds., Paul Parey, Hamburg, Germany, 2nd Ed., pp: 218-220.
39. Riad, N.H.A., H.A. Taha and Y.I. Mahmoud, 2009. Effects of garlic on albino mice experimentally infected with *Schistosoma mansoni*: A parasitological and ultrastructural study. Trop. Biomed., 26: 40-50.
40. Tzipori, S., 1983. Cryptosporidiosis in animals and humans. Microbiol. Rev., 47: 84-96.
41. Henriksen, S.A. and J.F. Pohlenz, 1981. Staining of Cryptosporidia by a modified Ziehl-Neelsen technique. Acta Vet. Scand., 22: 594-596.
42. Fayer, R. and L. Xiao, 2008. Cryptosporidium and *Cryptosporidiosis*. 2nd ed., Boca Raton: CRC Press, Taylor and Francis Group, Boca Raton, FL, USA.
43. Gornal, A.C., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177: 751-766.
44. Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chem. Acta, 31: 87-96.
45. Trinder, P., 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J. Clin. Pathol., 22: 158-166.
46. Dreux, C., 1977. Selected method. Analysis of human serum: assay of iron II. Method using bathophenanthroline Se-Iron II (Bathophenanthroline). Ann. Biol. Clin. (Paris), 35: 275-277.
47. Eden, A. and H.H. Green, 1940. Micro-determination of copper in biological material. Biochem. J., 34: 1202-1208.
48. SPSS 11, 2002. Statistical Package for Social Science, SPSS for windows Release 11.0.0, 12 June, 2002."Standard Version, copyright SPSS Inc., 1989-2002".
49. Göz, Y., N. Altuğ, N. Yüksek and C. Özkan, 2006. Parasites detected in neonatal and young calves with diarrhoea. Bull. Vet. Inst. Pulawy, 50: 345-348.
50. Osman, F.A. and A.H. Sadiek, 2008. Role of Cryptosporidial infection as a pathogen of neonatal calf diarrhea in Assiut Governorate. 13th Cong. Fac. Vet. Med. Assiut Univ.
51. Lassen, B., A. Viltrop, K. Raaperi and T. Järvis, 2009. *Eimeria* and *Cryptosporidium* in Estonian dairy farms in regard to age, species and diarrhoea. Vet. Parasitol., 166: 212-219.
52. Lemar, K.M., M.P. Turner and D. Lioyd, 2002. Garlic (*Allium sativum*) as an anti-Candida agent: a comparison of the efficacy of fresh garlic and freeze-dried extracts. J. Appl. Microbiol., 93: 398-405.
53. Bailey, J.M. and J. Erramouspe, 2004. Nitazoxanide treatment for giardiasis and cryptosporidiosis in children. Ann. Pharmacother., 38: 634-640.
54. Lallemand, M., A. Villeneuve, J. Belda and P. Dubreuil, 2006. Field study of the efficacy of halofuginone and decoquinate in the treatment of cryptosporidiosis in veal calves. Vet. Rec., 159: 672-676.
55. De Waele, V., N. Speybroeck, D. Berkvens, G. Mulcahy and T.M. Murphy, 2010. Control of cryptosporidiosis in neonatal calves: Use of halofuginone lactate in two different calf rearing systems. Prev. Vet. Med., 96: 143-151.
56. Naciri, M., R. Mancassola, P. Yvore and J.E. Peeters, 1993. The effect of halofuginone lactate on experimental *Cryptosporidium parvum* infections in calves. Vet. Parasitol., 45: 199-207.
57. Awadalla, S.A.M., 1996. Studies on enteritis in newly-born buffalo calves. Ph.D. Thesis, Fac. Vet. Med., Zagazig University, Egypt.
58. Sadiek, A.H. and G. Schlerka, 1996. Studies on rehydration therapy in diarrheic milk fed calves, Tierartztliche Umscahu., 51: 544-552.
59. Fadl-Alla, M.G.E., 1989. Clinical, hematological and biochemical studies on calves suffering from diarrhea. M.V.Sc. Thesis, Fac. Vet. Med., Cairo University, Egypt.
60. Malina, J.M., E. Rodriguez-ponce, O. Ferrer, A.C. Cutierrez and S. Herndez, 1994. Biopathological data of goat kids with cryptosporidiosis. Vet. Rec., 135: 67-68.
61. Mohga, S.A., 1994. Clinico-pathological and therapeutic studies on diarrhea in neonatal buffalo calves. 2nd Vet. Med. Cong., Zagazig.

62. El-Sebaie, A.H. and N.K. Hassan, 1980. Infectious bovine rhinotracheitis and virus diarrhea, virus infectious fattening. Assuit Vet. Med. J., 14: 247-248.
63. Anderson, B.C., 1981. Patterns of shedding of cryptosporidial oocysts in Idaho calves. J.A.V.M.A., 178: 982-984.
64. Tawfik, S.A., S.A. El-Dessouky and R.A. El-Ramady, 2004. Blood abnormalities associated with hair loss "Alopecia" in suckling calves. 7th Sci. Vet. Med. Zagazig Conf., Sharm El-Sheikh, Egypt.
65. Radostits, O., C. Gay, W.H. Kenneth and P.D. Constable, 2007. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10th Ed. Publisher: Saunders Ltd.
66. Youssef, M.M., S.M. Amin, L.M. Abou Samra, W.M. el-Gebaly, S.M. Hammam, E. ElSabaawy and A.M. Khalifa, 1992. A study on experimental cryptosporidiosis. J. Egypt. Soc. Parasitol., 22: 667-674.
67. Weldon, A.D., N.S. Moiseand W.C. Rebhun, 1992. Hyperkalemic atrial standstill in neonatal calf diarrhea. J. Vet. Intern. Med., 6: 294-297.
68. Nasr, M.Y.A., 1993. Nutritional disturbance among buffalo calves. Ph.D. Thesis, Fac. Vet. Med., Zagazig University, Egypt.