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EFFECT OF CRYTOSPORIDUIM PARVUM INFECTION ON THE HAEMATOLOGICAL AND BLOOD BIOCHEMICAL CHANGES OF BUFFALO CALVES WITH SPECIAL REFERENCE TO THE PREVALENCE OF INFECTION AMONG BUFFALOES

(With 7 Tables and 2 Figures)

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أثر العدوي بطفيل الكربتوسبوردیا بارفام علي صورة الدم والتغيرات البيوكيميائية في دم عجول الجاموس مع الإشارة إلي معدل الإصابة في الجاموس

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أجريت هذه الدراسة علي عدد ٢٩٠ من الجاموس المصري في بعض المزارع الخاصة بمحافظة الدقهلية علي مدار عام في الفترة من يناير إلي ديسمبر ٢٠٠٤، منها عدد ٢٥٠ عجل تتراوح أعمارها من يوم إلي ستة أشهر، ٤٠ حيوان بالغ "٤ – ٦ سنوات". وقد أظهرت نتائج الفحص الطفيلي بالدراسة أن معدل الإصابة بطفيل الكربتوسبوردیا كانت ١٩,٦٥% (٢٢,٤٠% في الحيوانات أقل من ستة أشهر، ٢,٥٠% للحيوانات البالغة) كما أظهر الفحص الإكلينيكي للحالات المريضة المصابة بالطفيل عدم وجود ارتفاع في درجة الحرارة مع وجود درجات متفاوتة من الإسهال وفقدان للشهية وضعف عام وعدم القدرة علي رضاعة اللبن علاوة علي انخفاض في الوزن الحي للحيوان وكذلك وجود جفاف بدرجات متفاوتة طبقا لمعدل الإصابة. تلاحظ أيضا أن أعلي نسبة إصابة كانت في العجول من عمر يوم حتي شهر (٣٢,٣٨%). هذا وقد اتضح أن أعلى معدل للإصابة كان في فصل الشتاء يليها الربيع والخريف والصيف (٤٨,٣٨%، ٢٣,٨٠%، ٩,٦٧%، ٧,٩٣%) علي الترتيب. وفيما يخص التغيرات البيوكيميائية فقد أوضحت الدراسة وجود نقص معنوي عال في مستوي البروتين الكلي، الألبيومين والجلوبيولين. كما أظهرت نتائج التحليل الكهربي لمكونات البروتين وجود نقص معنوي عال في جزيئات الجاما جلوبيولين عند مقارنتها بالمجموعة الضابطة نتيجة للضعف العام وعدم قدرة الحيوان علي الرضاعة في هذه الفترة مما أدي إلي نقص الأجسام المناعية اللازمة لمقاومة الأمراض. هذا وقد أوضحت الدراسة وجود نقص معنوي عال في كرات الدم الحمراء وتركيز الهيموجلوبين، وفي الجانب الآخر

تلاحظ وجود زيادة معنوية في العد الكلي لكرات الدم البيضاء وحجم الخلايا المصمتة بالإضافة إلي وجود نقص معنوي واضح في كل من سكر الدم والصوديوم والكلوريدات والحديد ، وعلي النقيض وجدت زيادة معنوية في مستوي كل من يوريا الدم والكرياتينين والبوتاسيوم. وقد تم استخدام مركب السلفا ديمدين ومحلول رنجر لاكتات بالإضافة إلي استخدام نيوالدايكلين لعلاج الحالات المرضية. وأوضحت الدراسة أن الحيوانات التي تم علاجها قد عادت كل معدلاتها الدموية إلي ما يقرب من مستواها الطبيعي بعد عشرة أيام من بدء العلاج.

SUMMARY

The present study was conducted on 290 buffaloes, 250 of them were newly-born calves, aged from one day to six months old, and 40 of them were adults, in the period from January to December 2004. These animals belonged to some private farms in El-Dakahlia governorate. The results of parasitological examination in the present study cleared that the infection rate of Cryptosporidium parvum was 19.65% (22.40% in animals less than 6 month age and 2.50% in adult ones). The clinical examination of the diseased animals infected with the parasite revealed no elevation in body temperature and presence of diarrhea of different degrees, anorexia, general weakness, unability for suckling milk with decreased of life animal body weight, also presence of variable state of dehydration with different degrees according to the infection rate. It was observed that the highest rate of infection was in calves aged from one day to one month old (32.38%), Highest rate of infection was in winter season followed by spring, autumn and summer (48.38%, 23.80%, 9.67% and 7.93%) respectively. Dealing with the biochemical changes, the study proved that there were significant decreases in total serum protein, albumin and globulin levels. The results of protein electrophoresis cleared that there was a high significant decrease in gamma globulins fraction when compared with control group as a result of general weakness and unability of the animals for suckling in this period which leads to decreased antibody needed for immunity against diseases. The study declared a high significant ($P < 0.01$) decrease in TRBCs and Hb. On the other side a significant ($P < 0.01$) increase in TWBCs and PCV in addition to presence of significant decrease in serum glucose, sodium, chloride and iron levels. On the contrary there were significant increase in blood serum urea, creatinine and potassium levels was evident. Sulphadimidine powder, ringers lactate solution in addition to New Diaclean were used for the treatment of the diseased

cases. The study revealed that all the treated cases showed normal levels in their blood biochemical parameters after 10 days of treatment.

Key words: *Buffalo calves, Cryptosporidium parvum, prevalence, biochemical changes.*

INTRODUCTION

Buffaloes are considered the main integral part of the farming system for small farmers in Egypt, where they constitute the most important sources of milk and meat production. The total number of buffaloes in Egypt is 1541690 (GOVS, 2004), while the number of buffalo calves in El-Dakahlia governorate was 154560 (Dakahlia Veterinary Records, 2004). Although buffaloes are susceptible to most infectious diseases that affect cattle, they have a sort of resistance against infection compared with other domestic livestock (Shalash, 1984).

Cryptosporidium parvum, an apicomplexan parasite of the mammalian gut epithelium in a wide range of hosts and transmitted by contamination of food or water with oocyst laden feces from an infected animal (Priest *et al.*, 2003).

Cryptosporidium parvum are minute extracellular parasite adhering to the brush border of enterocytes of the intestinal mucosa. It has the ability to accelerate and promote loss of absorptive function of cells. This mechanism could result in villous atrophy, impaired digestion and absorption. Thereby causing diarrhea in newborn animals (Kirkpatrick and Fanel, 1984). The diarrhea caused by such parasite is considered a major cause of health problems in cattle herds. So, every practical economical effort should be made to minimize the disease and mortality.

Enteric protozoal affections of buffaloes are considered as one of the most important problems which seriously affect the animal production. This is attributed to a lot of harms exerted by different types of enteric parasites including competition for host nutrients, destruction of host tissues, mechanical interference, as well as irritation and inflammatory reaction of the hosts tissue producing diarrhea (El-Sherif *et al.*, 2000). Moreover, parasites may also cause loss of blood, body fluids and plasma protein, impair absorption of digested nutrients and decrease weight gain of infested animals (Soulsby, 1982).

Cryptosporidiosis is an infectious disease primarily of neonatal farm animals caused by Cryptosporidium parvum. It is obligate

protozoan inhibiting the gastrointestinal tract of wide range of mammals causing diarrhea (Hill, 1990). Cryptosporidiosis infection causes considerable economic losses due to high morbidity among affected animals at the age of one day to one month old (Abdel-Salam *et al.*, 1993). Moreover numerous cases of zoontic transmission from animals to humans have been reported (Casemore, 1990). Due to the limited availability of effective drugs, the control of Cryptosporidiosis relies mainly on hygienic measures and good management (DeGraaf *et al.*, 1999). Therefore the present work aimed to study the prevalence of Cryptosporidium among buffalo calves, clinical signs of the disease, some blood biochemical constituents as an aids to diagnosis in diseased calves, relation between Cryptosporidial infection and colostral immunity in addition to performing treatment trial for diseased animals.

MATERIALS and METHODS

I- Animals:

A total number of 290 buffaloes were included in the present study, 250 of them were newly-born buffalo calves aged from one day to six months old and 40 of individuals were adults (4 – 6 years). All animals belonged to private farms in El-Dakahlia governorate. This survey was carried out in the period from January to December 2004. the animals were clinically examined for signs of health and disease, special attention to body temperature, mucus membranes, appetite, diarrhea, constipation, dehydration in addition to signs of gastrointestinal disturbances. All these examinations were performed with referencing to the method adopted by Smith (2000).

II- Samples:

1- Faecal samples were obtained directly from the rectum in a separate clean labled container.

2- Two blood samples were collected from Jugular vein via venoject system, from each examined calve, in a dry sterile centrifuge tubes. The first blood sample was collected with anticoagulant (sodium salt of EDTA) for haemogram picture and the second one was collected without anticoagulant for preparation of biochemical investigations.

III- Parasitoglogical examination:

1- Direct smear examination for detection of the parasite was carried according to Soulsby (1982).

2- Acid fast staining was done for detection of Cryptosporidum oocyst using modified Ziehl Neelson technique according to Henricksen and Phlenz (1981).

IV- Haematological and blood serum biochemical analysis:

1- Blood picture:

A- The total erythrocytic and total leucocytic cells counts (x 10^6 & $10^3/mm^3$) were determined according to Shalm *et al.* (1975).

B- The haemoglobin content (g/dL) and packed cell volume (%) were estimated after Coles (1986).

2- Blood serum biochemical analysis:

Both of total proteins, glucose, blood urea and creatinine levels in sera were estimated using test kits supplied by bio-Merioux. Determination of serum iron and copper levels in serum were done according to the method described by Allain and Maurous, (1979). Serum sodium and potassium were estimated photometerically by a flame photometer (corning Model 400 England ESSEX) using calibrating standard curve of sodium and potassium. Blood serum chloride level was estimated using chloride analyzer (Corning Model 925, England ESSex).

3- Blood proteins serum electrophoresis:

The proteins electrophoretic pattern was separated according to their respective electrical charges at pH 8.8 on a cellulose plate using both electrophoretic and electroendosmotic forces present in the system according to Alper (1974).

Treatment trials:

The principal lines of treatment for diseased calves are divided to:

1- Chemotherapy for Cryptosporidiosis:

Sulphadminidine powder was given in dose of 0.4 gm/kg. B.W. orally for 3-5 days.

2- Fluid therapy:

It is the most economic and effective when used as early as possible as Ringer lactate solution (El-Nasr Pharm Chem. Company). Bottle of 500 ml given by parentral therapy. The dose and route depended on degree of dehydration (Hunt, 1985).

3- Treatment of digestive tract lesions:

Astringents and adsorbents as New-Diaclean (Avioc Amman Jordan) sachet 12 gm composition: Neomycin 400 mg, Furazolidone, 400 mg, Sulphaguandine 400 mg, Kaolin 400 mg, pectin 400 mg, Bismuth 2000 mg, vitamin A 80000 IU.

Dose: ½ sachet for calf was given orally with 100 ml tap water twice daily for 3-5 days.

V- Statistical analysis:

All data were subjected to statistical analysis according to Snedecor and Cochran, (1982) by using a computer program on a way completely randomized, analysis of variance test "F test" treatment means were then compared by the least significant difference test "LSD" at 0.05, 0.01 and 0.001 levels of probability.

RESULTS

Concerning the clinical investigation of the infected buffalo calves with cryptosporidium parvum, infected animal shows yellowish greenish or clay coloured profuse watery diarrhea, anorexia, colicy pain, normal body temperature and reduced milk. With the progression of the disease, animals dehydrated with evidence of lying down posture.

The results are recorded in tables (1-7) and figures (1 & 2).

Table 1: Prevalence of Cryptosporidiosis in newly-born buffalo calves and adult buffalo.

Animal status	No. of examined animals	+ve	%
Buffalo calves	250	56	22.40
Adult buffalo	40	1	2.50
Total	290	57	19.65

Table 2: Prevalence of Cryptosporidiosis in diarrhoeic and non diarrhoeic newly-born buffalo calves.

Animal status	No. of examined animals	+ve	%
Diarrhoeic calves	210	58	27.19
Non diarrhoeic calves	40	4	10.00

Table 3: Prevalence of Cryptosporidiosis in relation to age.

Age	No. of examined animals	+ve	%
> month	105	34	32.38
1-3 month	85	16	18.82
3-6 month	60	6	10.00
Adult (4 – 6 years)	40	1	2.50

Table 4: Seasonal incidence of Cryptospordiosis in examined animals.

Season	No. of examined animals	+ve	%
Winter	62	30	48.38
Spring	63	15	23.80
Summer	63	5	7.93
Autumn	62	6	9.67
Total	250	56	22.40

Table 5: Mean values of serum protein electrophoresis in healthy and naturally cryptosporidial infected newly-born buffalo calves before and 10 days post treatment.

Item / Parameters	Control healthy claves	Cryptosporidial infected calves	
		Before treatment	*Post treatment*
Total proteins (g/dl)	7.68 ± 0.28	6.07 ± 0.35**	7.33 ± 0.46N.S
Albumin (g/dl)	3.61 ± 0.28	2.43 ± 0.24**	3.19 ± 0.21N.S
Globulin (g/dl)	4.07 ± 0.12	3.64 ± 0.06**	4.14 ± 0.17N.S
A/G ratio	0.89 ± 0.08	0.66 ± 0.11N.S	0.77 ± 0.09N.S
α-globulins (g/dl)	1.15 ± 0.10	1.79 ± 0.30N.S	1.12 ± 0.15N.S
β-globulins (g/dl)	1.35 ± 0.09	0.93 ± 0.14*	1.24 ± 0.21N.S
δ-globulins (g/dl)	1.57 ± 0.15	0.92 ± 0.08**	1.78 ± 0.23N.S

*: significant at P < 0.05 **: highly significant at P < 0.01 N.S: non significant

Table 6: Mean values of some haemogram parameters in healthy and naturally cryptosporidial infected newly-born buffalo calves before and 10 days post treatment.

Item / Parameters	Control healthy claves	Cryptosporidial infected calves	
		Before treatment	*Post treatment*
TRBCs ($x10^6$/UL)	7.95 ± 0.19	6.91 ± 0.18**	7.93 ± 0.20N.S
Hb (g/dl)	11.59 ± 0.23	10.17 ± 0.28**	11.41 ± 0.24N.S
PCV (%)	36.68 ± 0.42	39.83 ± 0.69**	36.13 ± 0.65N.S
TWBCs ($x10^3$/UL)	9.57 ± 0.17	10.40 ± 0.22*	9.89 ± 0.28N.S

*: significant at P < 0.05
**: highly significant at P < 0.01
N.S: non significant

Table 7: Mean values of some blood serum biochemical parameters in healthy and naturally cryptosporidial infected newly-born buffalo calves before and 10 days post treatment.

Item / Parameters	Control healthy claves	Cryptosporidial infected calves	
		Before treatment	*Post treatment*
Glucose (mg/dl)	72.31 ± 1.29	61.53 ± 2.15**	71.47 ± 2.46N.S
Blood urea (mg/dl)	23.36 ± 0.75	27.49 ± 0.99**	22.75 ± 0.59N.S
Creatinine (mg/dl)	0.89 ± 0.01	0.95 ± 0.02*	0.86 ± 0.01N.S
Sodium (m.Eq/L)	133.14 ± 2.09	125.47 ± 1.78*	131.69 ± 1.52N.S
Potassium(m.Eq/L)	4.49 ± 0.25	5.87 ± 0.23**	4.47 ± 0.18N.S
Chloride (m.Eq/L)	96.35 ± 0.38	94.24 ± 0.64*	95.87 ± 0.51N.S
Iron (µg%)	254.57 ± 3.31	236.39 ± 3.98**	251.72 ± 5.37N.S
Copper (µg%)	85.412 ± 3.34	76.51 ± 3.14N.S	82.74 ± 3.81N.S

*: significant at P < 0.05 **: highly significant at P < 0.01 N.S: non significant

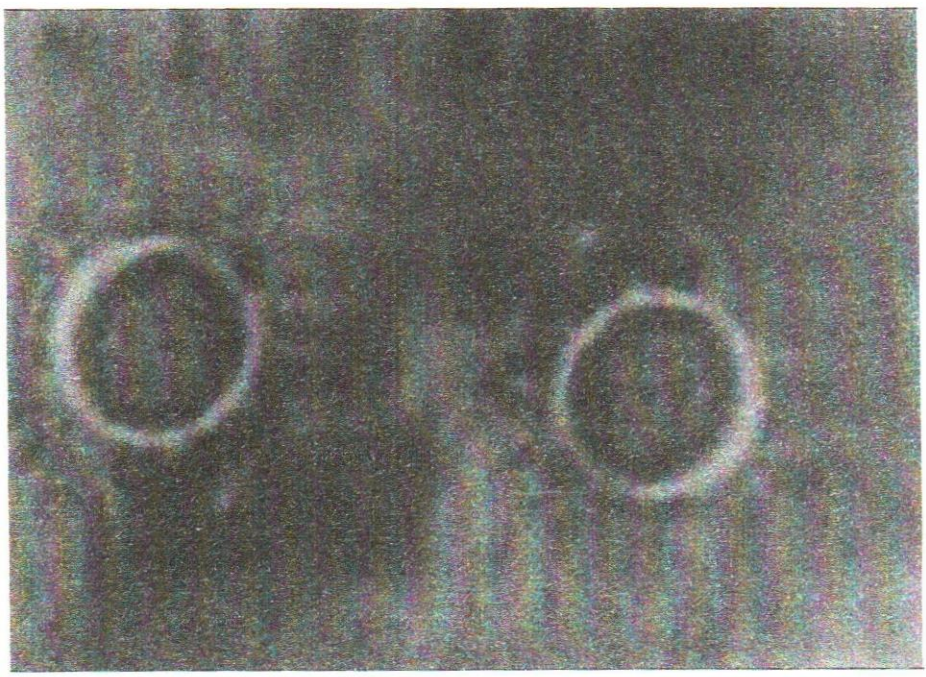

Fig. 1: Cryptosporidium parvum oocyst stained with modified Ziehl-Neelson X1250.

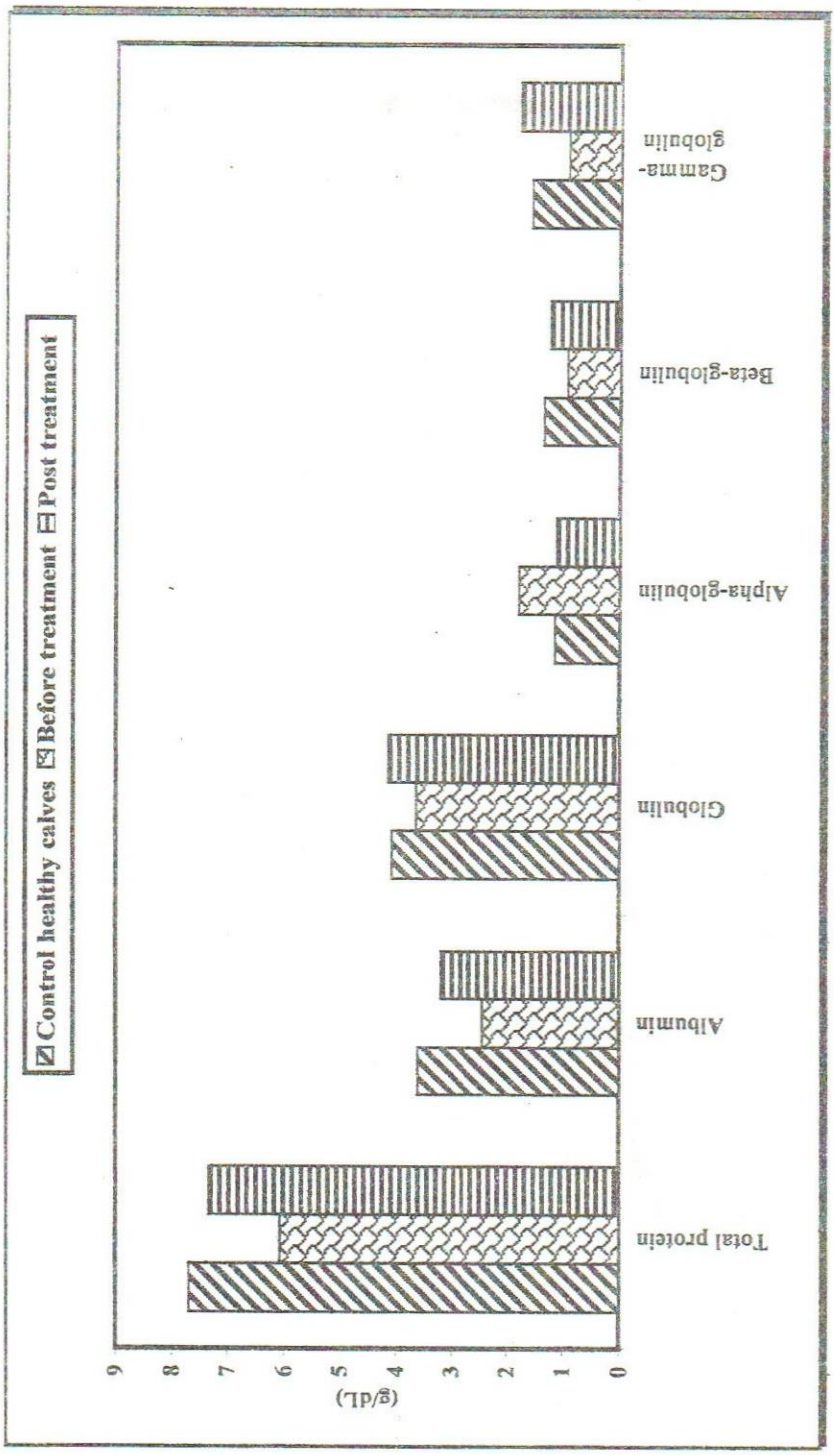

Figure 2 : Serum protein electrophoresis of control healthy and naturally cryptosporidial infected newly born buffalo calves before and 0 days post treatment

DISCUSSION

The yellowish greenish or clay coloured profuse watery diarrhoea, anorexia, colicy pains, normal body temperature, reduced milk suckling with the progression of the disease and the occurring dehydration of lying down posture of infected calves with cryptosporidia agreed with El-Khodery, (1996) and El-Sherif *et al.*, (2000). Dealing with the prevalence of cryptosporidiosis, cryptosporidium oocysts which were detected in the present study 56 (22.40%) of the examined calves and 1 (2.50%) of the examined adult buffaloes (Table 1), these results coinside with El-Khodery, (1996) and El-Sherif *et al.*, (2000). Bendali *et al.*, (1999) detected cryptosporidium oocysts in 15.6% of the examined cases. These differences may be attributed to the differences in severity of infection, breed of calves and hygienic measures.

Concerning the diarrhoeic and non diarrhoeic calves the occurring prevalence of infection which were 58 (27.19%) and 4 (10%) respectively as shown in Table 2. These findings nearly came in agreement with El-Khodery, (1996), who stated that the ratio was 41.82% in diarrhoeic calves and 5.96% in non diarrheic individuals.

Meanwhile, Bjorkman *et al.*, (2003) detected cryptosporidium oocysts in 11% of the examined diarrhoeic claves and 5% of the examined healthy ones. These difference may be attributed to the breed, severity of infection and season of examination.

Eventually animal age plays a great role in calf susceptibility to cryptosporidium infection in the present study. It has been observed that calves less than one month recorded the highest degree of infection 34 (32.38%) followed by calves 1-3 months 16 (18.32%), then calves 3-6 months, 6 (10.00%) but adults recorded 1(2.50%) as illustrated in Table 3. These results revealed that cryptosporidiosis is a disease of young ages and gradual decrease of infection rate occurred with age progression. These findings were emphasized by Busto *et al.*, (1998) who reported that cryptosporidium prevalence was high in suckling than in dairy cows, Meanwhile, O'Hanley *et al.*, (1999) stated that cryptosporidium was an important pathogen when calves were less than one month old. Uga's *et al.*, (2000) reported that 93% of calves more than 30 days old are positive to cryptosporidium. Also, El-Sherif *et al.*, (2000) found that the highest degree of infection was in calves > 1 month and then gradually decrease with age to reach 0% in calves over 4 month old, while Huetink *et al.*, (2001) stated that shedding of cryptosporidium spp. oocysts was found in all ages, but peaked in calves

1-3 weeks old. Finally, Castro Hermida *et al.*, (2002) declared that the prevalence of cryptosporidium parvum in calves of less than 3 weeks old was 47.9%. The differences in the ratio from our study may be related to season in which the calves examined.

The highest rates of infection in calves were in winter, spring and autumn (48.38%, 23.80% and 9.67% respectively), while summer recorded the lowest rate (7.93%) as shown in Table 4. Bendali *et al.*, (1999) came in accordance with these findings in that the highest incidence of cryptosporidiosis was in winter and with Lefay *et al.*, (2000) who found that the lower infection rate was in summer, but El-Sherif *et al.*, (2002) found that the highest rate of infection was in winter and the lowest rate was in autumn. These differences could be attributed the differences between localities and weather (humidity and temperature).

Protein electrophoresis:

Concerning the mean values of blood serum total protein, albumin and globulins levels, Table (5) & Figure (2) which revealed that there was a high significant ($P < 0.01$) decrease in parasitized calves in comparison to healthy ones. These results were supported by similar findings recorded by Mohga, (1994), Awadalla, (1996) and nearly agreed with those results previously obtained by El-Khodery *et al.*, (2002) in lambs. The significant reduction in total proteins and albumin in diseased calves could be attributed to poor absorption of nutrients and excessive protein breakdown and loss of albumin (Molina *et al.*, 1994), while decreased globulins levels may be due to marked depression in gamma globulins fraction associating the diseased condition (El-Sebaaie and Hassan, 1980).

Regarding to the mean values of serum globulins fraction (Table 5 & Figure 2), the significant ($P < 0.05$) and high significant ($P < 0.01$) decrease in the level of Beta and gamma globulin fractions respectively when compared with apparently healthy calves came in accordance with those recorded by Awadalla, (1996) in calves and Nassif *et al.*, (2002) in lambs and goat kids. The marked reduction in Beta and gamma globulins levels could be attributed to failure of calves to receive adequate quantity and quality of colostrum after birth due to diarrhoeic condition (Ahmed, 1990).

Haematological parameters:

In Table 6 the highly significant ($P < 0.01$) decrease in the mean values of TRBCs count and Hb in diseased calves agreed with those previously reported by Awadalla, (1996) in case of Hb content in buffalo

calves and Nassif *et al.*, (2002) in lambs and goat kids. This obvious reduction in TRBCs count and Hb mean values may be related to the incidence of dehydration and haemoconcentration arised from diarrhea. Concerning the mean value of PCV. Table (6) showed a highly significant ($P < 0.01$) increase in diseased calves than control healthy ones. Such increase may be referred to the decrease in plasma volume resulting from excessive loss of body fluid in the faeces of diarrhoeic calves, in addition to inadequate intake of milk and fluids during enteritis (Mohamed, 1986 and Fadl-Alla 1989).

The significant ($P < 0.05$) increase in cryptosporidial infected calves of total leucocytic count when compared with healthy ones (Table 6) could be declared according to Molina *et al.*, (1994) who said that the infection with cryptosporidia or any protozoa like parasite eventually predispose for bacterial invasion which subsequently leads to an increase in the total leucocytic count.

Biochemical analysis:

In Table (7), the high significant ($P < 0.01$) decrease in the glucose level when compared with healthy calves agreed with those obtained by Mohamed, (1986) and Tawfik *et al.*, (2004). This could be attributed to lack of glucose absorption from damaged intestine particularly in cryptosporidiosis that cause villous atrophy which impairs digestion and absorption resulting in diarrhea (Anderson,1981).

Concerning mean values of blood serum urea nitrogen and creatinine, (Table 7) revealed a highly significant ($P < 0.01$) and significant ($P < 0.05$) increases respectively, came in accordance with those reported by Mohga, (1994). The noticable increase in serum blood urea level may be attributed to deficient renal blood flow and reduced urine formation in calves with fluid deficit in an attempt to conserve body fluid (Fisher, 1965). On the other hand the significant increase in serum creatinine level in diseased calves can be considered to be due to the high levels of all metabolic waste materials which include creatinine resulted from reduced amount of urine in an attempt to conserve body fluids (Walt, 1965).

The significant decrease in serum sodium and chloride level ($P < 0.05$) when compared with those of control healthy calves agreed with Fadl-Alla, (1989) and Mohga, (1994) who attributed this decrease to the loss of large amount of sodium and chloride ions with intestinal secretions associated with diarrhea. With regard to the mean value of blood serum potassium level (Table 7) the occurring high significant ($P < 0.01$) increase when compared with apparently healthy calves.

These results were supported by the findings of Mohamed, (1986) and Tawfik *et al.*, (2004). This obvious increase in serum potassium level 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 (Youssef *et al.*, 1992).

The insignificantly changes of copper level in blood serum of cryptosporidial infected calves if compared with apparently healthy ones, while the high significant ($P < 0.01$) of iron decrease when compared with healthy claves, were confirmed by the results reported by Nasr, (1993), who attributed this to be due to the decrease in Beta globulin level which has specific mineral binding capacity or to the anorexic condition of the diarrhoeic calves.

Chemotherapeutic trials:

The perfect therapy was based on the changes in haematological and biochemical parameters. Well marked clinical and laboratory results were obtained after the treatment of the diseased claves using sulphadimidine powder in a dose of 0.4 gm/kg. B.W orally for 3-5 days and using Ringer Lactate solution (4-4.8 litres) given by subcutaneous and intravenous routes, in addition to using New Diaclean in a dose of ½ sachet for calf per os with 100 ml tap water twice daily for 3-5 days. The haematological and biochemical parameters of blood and serum returned in general to their normal values at the end of treatment as shown in tables (5-7) and Figure (2). These results were agreeable with Awadalla, (1996).

Accordingly it could be concluded that, cryptosporidium is an enteropathogen of neonates calves, has the ability to induce diarrhea and gradually decreased with age, the adults may harbour the parasite without any clinical symptoms. In addition to the disease greatly affected by managemental system and has a relationship with seasonal variations and has an adverse effect on blood and biochemical parameters of the infected newly-born calves, where gamma globulin fraction greatly reduced. So good colostral transfer is highly efficient for prevention of infection as early as possible specially the first 24 – 36 hours.

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