Prevalence of Cryptosporidium parvum with oxidative stress and antioxidant status in sucker cattle calves suffering from diarrhea.

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Abstract:

The study was carried on 160 newborn calves (140 calves suffering from diarrhea and 20 calves, clinical and laboratory healthy and used as a control group),classified according to age into three group; group A(2day-1month),group B (1month- 2months) and group C(2months-3months),all calves selected from three different area according to the source of water, area 1(Deseret area),45 calves and used artesian water, area 2(Sewage area),50 calves and used sanitation water and area 3(Village area),45 calves and used Nile water. The study aimed **to determine the prevalence of C. parvum,** effect of water source on the prevalence of infection and summarize the cellular oxidant and antioxidant systems in newborn calves naturally infected. Fecal and blood samples were collected from all calves under the study for analysis ,the result revealed high percent of infection between calves(65%,44.44% and 20%) in area 2,3 and 1,respectively but in relation to age the result revealed that group A(26.66%,80.55% and 54.285) fallowed with group B(11.11%,16.66% and14.28%)in area 1,2 and 3 respectively, while group C revealed infection in area 2 only in ratio of 12.5%.Oxidative stress indicators revealed that highly significant increase in Malondialdehyed (2.99±0.11)with highly significant decrease in antioxidant parameters (Catalase and Superoxide dismutase enzymes),0.88±0.66 and 51.8±16.4,respectivelly.

Keyword:- Cryptosporidium parvum, Neonatal calves, Oxidative stress

Introduction

Infectious diarrhea of neonatal calves is common problem through the worldwide, and are leading causes of calves' death during their first weeks of life. A wide range of causative infectious agents may be involved in this pathology. In general, the main causes are bacterial infections, although viral and protozoan agents can also infect the animals concomitantly or exclusively within the 10-14 days of age. It has been reported that calves infected with the protozoan Cryptosporidium spp. are usually asymptomatic and only stressed animals or individuals with a concomitant pathology show clinical manifestations of the disease named cryptosporidiosis (Blowey and Weaver, **2003).Cryptosporidium parvum** has been identified as the second most common infectious agent in outbreaks of diarrhea (Fayer andUnger,1986 andAngus,1989).C. parvum is non-host specific and can easily be transmitted from one species to another. Infected calves and lambs pass up to 10^{10} oocytes by the feces between days 4 and 14 post infection.(Blewett, 1989) . Unlike other coccidian oocytes, C. parvum oocytes are fully sporulated and ready to initiate infection upon excretion (Current and Garcia, 1991). These oocytes were reported to contaminate drinking water resources (Modare et al,

1987).Contaminated drinking water may be a source of human cryptosporidiosis (D Antonia et al, 1985 and Hayes et al, 1987). **Cryptosporidium parvum** belongs to a ubiquitous and diverse group of intracellular apicomplexan parasites of both human and veterinary importance; it is an intracellular protozoan parasite causing gastrointestinal disease and diarrhea many vertebrates including humans, farm animals (cattle, sheep, goats, horses, chickens and turkeys), pets (dogs and cats), laboratory animals (rats and mice) and wild birds, reptiles and fishes (Gomez-Couso et al., 2005). In most of them, Cryptosporidium spp. grows and multiplies in the microvillus borders of the enteric epithelium cells. The parasite may additionally infect other tissues such as the respiratory and renal epithelia, especially in immune-compromised humans (Mercado et al., 2007; Fayer and Xiao, 2007).When oocytes' are ingested by a suitable host; the endogenous phase of the parasite life cycle begins by invasion of target cells. This biological process includes schizogony, gametogony, fertilization, and sporogony(Fayer and Xiao.,2007).The sporulated oocyte is the only exogenous stage in the life cycle of the parasite and being excreted with the feces of an infected host (Fayer and Xiao.,2007) is highly relevant for the diagnosis of cryptosporidium infection. It is eliminated in great quantities with fecal material and is capable of survival for long periods in the environment (Neira, 2005; Fayer and Xiao, 2007).The calf most often recovers spontaneously within 1-2 weeks even though there is a large variation between individuals in how they respond to and recover from infection but concomitant infection with other enteric pathogens can aggravate the clinical signs and prolong the duration of disease (O'Donoghue 1995).Finding oocytes in diarrheic feces is indicative of C. parvum being the cause of the disease, where infected calf can shed millions of oocysts during the first two weeks, which ensures efficient dissemination of the parasite (Fayer et al. 1998, Uga et al. 2000).

Cryptosporidium infection is most frequent in calves less than one month of age, clinically characterized by varying degrees of diarrhea. In immune-compromised humans it is a severe disease, in cattle is mainly caused by C. parvum, difficult to treat and can be fatal if extreme dehydration occurs (Sunnotel et al., 2006).Presently, there is no specific anti-parasitic chemotherapy for the treatment of Cryptosporidium infections (Caccio and Pozio, 2006). The disease in calves has been studied in many countries, with prevalence ranging between 2.4% and 100% (Fayer and Xiao, 2007). Cryptosporidium parvum causes diarrhea and sometimes mortality in a broad range of mammals, while in calves, the economic impact of cryptosporidiosis is considerable and comparable to that of rotavirus infection. It has been identified as the second most common infectious agent in outbreaks of diarrhea (Angus, 1989).Also is nonhost specific and can easily be transmitted from one species to another. Infected calves and lambs pass up to 10^{10} oocytes by the feces between days 4 and 14 post infection (Blewett, 1989).Unlike other coccidian oocyte, C. parvum oocytes are fully sporulated and ready to initiate infection upon excretion (Current and Garcia, 1991).These oocytes were reported to contaminate drinking water resources (Modore et al, 1987). Contaminated drinking water may be a source of human cryptosporidiosis (Hayes et al, 1987).Cryptosporidium spp., in contrast to other apicomplexan parasites, has a monoxenous life cycle that takes place in the gastrointestinal tract of the host. Where during the encystation, four infective sporozoites are released from the oocyte, glide over the intestinal cells until they invade the cell using the apical complex and after infection the parasite develops at the apical surface in the host cell, thus the parasite is in an intracellular but extra- cytoplasmic state. Cryptosporidium spp. is protected from the gut environment and use nutrients via an Apicomplexa-unique feeder organelle. Uniquely

amongst other apicomplexan parasites, cryptosporidium spp. lacks an apicoplast and also has lost the mitochondrial genome and most of its functions (Keithy et al, 2005).

Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism. At low to moderate concentrations, they function in physiological cell processes, but at high concentrations, they produce adverse modifications to cell components, such as lipids, proteins and DNA (Valko et al, 2006). The shift in balance between oxidant/antioxidant in favor of oxidants is termed "oxidative stress." Oxidative stress contributes to many pathological conditions, including cancer, neurological disorders,(Jenner,2003),atherosclerosis, hypertension, ischemia/perfusion(Dhalla et al,2000) ,diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease(Asami et al,1997)and asthma (Andreadis,2003).

Aerobic organisms have integrated antioxidant systems, which include enzymatic and no enzymatic antioxidants that are usually effective in blocking harmful effects of ROS. However, in pathological conditions, the antioxidant systems can be overwhelmed, but only limited information is present of the antioxidant enzymes in Cryptosporidium species, while the enzymes glutathione transferees, glutathione reeducates and glutathione peroxidase seem to be absent in C. parvum, some SOD activity has been detected (Entrala et al,1997). Further, it has been shown that the parasite contains and synthesizes GSH and possesses a thioredoxin peroxidase that could be important for detoxification and thereby protection against ROS (Joung et al, 2011 and Yoon et al, 2012).

In this article, we aimed to determine the prevalence, effect of water source on the prevalence of infection and summarize the cellular oxidant and antioxidant systems in newborn calves naturally infected with cryptosporidium parvum.

Materials and methods
A-Study area
The study was conducted three different localities in Assuit governorate, according to water source as the follow, animals living in the desert areas (artesian wells), animals living in sewage area(with sanitation water), and animals living in villages (Nile water).Fig 1.

B-Animals
The present study was carried on 160 newborn calve of both sexes, age ranged from 2 days to 3 months, calves reared in three different localities according to the source of water, area (1), 45 newborn calf suffered from diarrhea, reared on artesian well in deserted area, area (2),50 newborn calve suffered from diarrhea, reared on sanitation water in area of sewage and area(3),45 newborn calf suffered from diarrhea, reared on Nile water in villages area. Also these calves classified according to age into three groups, group (A), 2 day up 1 month, group (B), 1up to 2 month and group (c), included calves aged from 2 month up to 3 months. Twenty newborn calve, clinical and laboratory healthy and used as control group.

C-Sampling
1- Fecal samples
The samples were obtained directly from the rectum by scrubbing the rectum of diarrheic calves, samples were collected in plastic tube containing formaldehyde 10% (Weitze

andTossara,1989),simples identified by collection date, calf age and number, samples' were temporally stored in an ice tank and taken to the laboratory of parasitology (Casemore,1991).

2- Blood sample

5ml blood without anticoagulants, centrifuged to obtained serum and stored at 5 C until be used for biochemical analysis.

D-Clinical examination of animals

the examination of clinical healthy and diarrheic calves were carried out carefully using the methods described by (Radostites et al, 2007).

K-Isolation and identification of causative agents

1- Parasitological examination

Processing of fecal samples consists of filtering the samples through a filter and centrifuged at1500xg for 15 minutes, for zahiel-Neelsen staining, the sediment of samples was suspended in 500pl, fifty microliters of the suspension was spread on glass slides(3 slides at least for each sample), air dried, staining with zahiel-Neelsen stain and microscopically screened for cryptosporidium oocytes (Casemore,1991).A sample was consider positive for cryptosporidium if at least one oocytes was detected upon direct microscope (100x) examination. Fig 2

2- Bacteriological examination

Sterile swabs for bacteriological examination were immediately inoculated on Carry and Blairs transport medium and cultured on selective and differential culture media at 37c for24 hours and the isolated colonies was then identified, isolated colonies from Mac-Conkys agar plate were examined to be either lactose fermenting or nonlactose fermenting. Lactose fermenting colonies appear to be rose pink in color and non-lactose fermenting appeared as pale yellow colonies. Isolated colonies were examined by gram staining (gram negative bacilli).

3- FASTest® D4T cattle test kits

All samples were tested with FASTest® D4T cattle test kits (MegaCor Diagnostik GmbH, LochauerstraBe 2xA - 6912 - Horbranz, AUSTRIA). The test kit included 4 rapid immunechromatographic test strips for the detection of cattle BVC, RV, C. parvum and E.coli-K99 (F5).The tests were carried out according to the manufacturer's guidelines.

E-Biochemical analysis

1. MDA Analyses

MDA levels were determined by colorimetric method, according, Ohkawa et al, (1979), based on thiobarbuturic acid (TBA) reactivity. According to this method, a stable red matter giving absorbents at 534 nm, where thiobarbuturic acid (TBA) react with malondialdehyde (MDA) in acidic medium at temperature of 95C for 30 minutes to form thiobarbituric acid (TBA),reactive product which spectrophotometric determined, as an indirect marker of oxidative stress in terms of TBARS (thiobarbituric acid reactive substances).

2. SOD Analyses

SOD analyses were performed according to the method of Podczasy and Wei (1988).

Briefly, 50ul of serum, 75mMof tris-HCL buffer, 30mM of EDTA and 2mM of pyrogallol

were added. An increase in absorbance was measured spectrophotometric at 420nm for 30 minute activity of SOD is expressed as U/ml of serum.

3. Catalase Analyses;

Catalase analyses were performed according to the method of Aebi, H (1984). The reaction mixture contained (in 1 ml): 10 mm H2O2, 50mm potassium phosphate buffer, pH 7±2. The decrease in OD was followed at 240 nm for 1 min.

4. Statistical analysis;

Investigated calves from both cattle and buffalo species were divided into three groups (according to age) for analysis: 2day-1month old, 1-2 month old, 2-3 months old. The age categories were selected based on the pathophysiology of Cryptosporidium. Parvum and the age groups that have been previously described (de la Fuente et al.,1998).The age distributions of calves as well as the number and prevalence of causative agent in different age and area groups were compared in both cattle and buffalo calves using chi-square (SPSS, 16.0).

Results

A total of 140 diarrheic calves (cattle and buffalo calves),aged from 2 day-3 months were collected from 3 different localities according to the source of water [Area 1(45), artisan well water, Area 2 (50),sanitation water and Area 3(45),Nil water], the calves in each group divided into three groups according to age (A,B and C),2 day-1 month,1-2 month and 2-3 months espectively. Twenty healthy calve (clinical and laboratory healthy), from the different area under the study and as a control calves, their number was 6, 8 and 6 calves from area 1, 2 and 3 respectively (Table1).Three fecal sample from each animal at least for microscopically and onfirmatory test (Fast kites test), where positive calves studied for oxidative stress.

The finding of the study revealed that, the majority of calves infected with cryptosporidium parvum with the relation of area were in area 2,3 and 1 respectively, where the result were 31 out of 50 (65%),20 out of 45 (44.44%) and 9 out of 45 (20%) respectively, Table 2.

The findings of this study with relation to age showed that C. parvum is commonly found among group (A), 2 day-1 month old calves (8/30 (26.66%),29/36(80.55%) and19/35 (54.28%) in the different area 1,2 and 3 area respectively, while among group (B),1-2 months, the result were 1/9 (11.11%),1/6 (16.66%) and 1/7 (14.28%)in area 1,2 and 3 respectively, and group (C),2-3 months, revealed 1/8 (12.5%) within area 2 only Table3.

The positive calve for cryptosporidium may be mixed with other infection as showed in table4, where 3/9,11/31 and 4/20 were infected with cryptosporidium parvum with other pathogen in area 1, 2 and 3 respectively, the commonly pathogen present ,were, bacteria (E. coli, Salmonella) and/or virus, (Rota or corona). The clinical examination showed that calves infected with cryptosporidium parvum, suffer from a slight rise in temperature, watery diarrhea with foul odor, straining and may be accompanied by some blood points, Table 5.

The result of oxidative stress of cryptosporidium parvum infection in suckling calves illustrated in table 6,indicated highly significant increase in malandealdehyde(MDA) and highly significant decrease in both catalase and superoxide dismutase enzy

Fig 1; Showed the type of three localities under the study.
Fig 2; Direct smears shows spherical to oval pink color oocytes of cryptosporidium.
Parvum with Zahiel Neelsen stain.

Age. Localities	Group A (days-1month)	Group B (1month-2months)	Group C (2months-3months)	Total
Area 1(deserted area)	30	9	6	45
Area 2 (Sewage area)	36	6	8	50
Area 3 (villages area)	35	7	3	45
Total	101	22	17	140

Table 1;The number of diarrheic calves (under the study) from three localities in relation to age.

Animals	Number of diarrheic calves	Number of positive calves			
		Zehiel-Neelsen test(Z)		Fast test kites(F)	
Localities	N	N	%	N	%
Area 1(deserted area)	45	9	20	9	20
Area 2 (Sewage area)	50	31	65	31	65
Area 3 (villages area)	45	20	44.44	20	44.44

Table 2; Number of positive calves to cryptosporidium parvum in three different localities of study by two different tests.

| Localitie | Area 1 | | | | Area 2 | | | | | Area 3 | | | | |
Age \	N	Z P	%	F P	%	N	Z P	%	F P	%	N	Z P	%	F P	%
Group A	30	8	26.66	8	26.66	36	29	80.55	29	80.55	35	19	54.28	19	54.28
Group B	9	1	11.11	1	11.11	6	1	16.66	1	16.66	7	1	14.28	1	14.28
Group C	6	-	-	-	-	8	1	12.5	1	12.5	3	-	-	-	-
Total	45	9	20	9	20	50	31	62	31	62	45	20	44.44	20	44.44

Table 3; Number of positive calves in different three localities in relation to age.

Agent. Localities	C. parvum	(Mixed infection)	Total
Area (1)	6	3	9
Area (2)	20	11	31
Area (3)	16	4	20

Table 4; Number of diarrheic calve infected with Cryptosporidium parvum (single infection) or cryptosporidium with other infection (Mixed infection).

Animal Clinical signs	Positive calve	Healthy control calve
Temperature	38-39.5C	38-38.5C
Fecal consistency	Watery	Semisolid
Tensmus	Present tensmus	Absent
Blood with feces	May be found	Absent

Table 5. Indicated the most clinical signs in calves infected with Cryptosporidium parvum.

Animals Parameters'	Control calves	Infected calves
Malondialdehyed (MDA mmol/mT[1])	1.79±0.03	2.99±0.11***^
Catalase (u/mT[1])	2.82±0.04	0.88±0.06***^
Superoxide dismutase (SOD, u/mT[1])	138.6±14.4	51.8±16.4***^

Table 6; Oxidative and antioxidant parameters in both control and infected calves.

Discussions;

Diarrhea is a leading cause of economic losses to calves industry and major cause of calf mortality and morbidity during the first few weeks of life in most countries (Radostits et al., 2007). Defining the map of calve diarrhea-causing enter -pathogens allows for application of effective and targeted preventative measures. The objective of the current study was to determine the prevalence of Cryptosporidium .Parvum associated with neonatal calf diarrhea among both cattle and buffalo calves. Immune-chromatographic rapid tests (FASTest® Strips) the use of rapid tests was recommended with standard methods in calves with acute diarrhea where the specificity might be of higher importance than the sensitivity, which could be increased by testing on a herd basis (Luginbuhl et al., 2005).Bazeley, 2003, said that, microbial infections represent the main cause of neonatal diarrhea calves. This notion was contrast with the current study, where the majorly of investigated cases of calve diarrhea was proved due to parasitic agents and some cases may be due to mixed infection. In recent years, concern has increased regarding the potential public health hazard of water supplies contamination by C. parvum. Dairy herds are one of the potential sources of this parasite (Angus, 1989). The high percentage of positive samples (45% of analyzed animals and 84.6% of visited farms) showed the high occurrence of the parasite and possible environmental contamination due to the infected animals. Results reported in different localities show values ranging from 20% to 65% of properties contamination and high prevalence in sewage area, villages and desert area on the fallowing in ratio of 65%, 44.44% and 20% respectively, where that may be attributed to the pressure of water source contamination.

In this study we detected C. Parvum in the majority of calve aged 2day-1 months but also detected in 1 calves aged 1-2 months and 2-3 months, this result coincide with (Silverlas et al. 2013 and Bjorkman et al,2015). This could be attributed to an effect of a lower infection pressure in sucker herds resulting in calves getting infected later. Oxidative stress is created as the result of insufficient antioxidant enzyme asset or overproduction of free radicals in the body. Free radicals and lipid peroxidation has detrimental effects to the cell (lobo et al,2010) MDA a product of lipid peroxidation is an important indicator of oxidative damage of cell membrane as it is the most abundant aldehyde formed (Petry et al,2010). Our findings indicated highly significant increase in the level of MDA with highly significant decrease in level of catalase and superoxide dismutase enzyme. This may be attributed to the very abundant nature of the enzyme as antioxidant, where superoxide dismutase (SOD) and catalase enzyme are a component of the compensatory reflex of the metabolism to oxidative damage targeting to neutralize the free radicals (Daudelin et al,2011).

Conclusion,

In conclusion C. parvum was reported as the most frequently encountered causative agent among diarrheic cattle and buffalo calves while other enter pathogen infection seemed to be of minor importance in the investigated different area. The higher rate of C. parvum infection was recorded among the calves reared in area with contaminated water as the fallowing, sewage area with sanitation water ,villages area with Nile water and desert area (65%,44.44% and 20%) respectively. Since C. parvum appears to be a common infection on dairy farms with water contamination and is associated with scouring in young calves (2day-1 months), but other aged calves may be infected with very low degree. For this attention for the hygiene of water source needs to reducing the frequency and intensity of this infection in dairy calves.

We coincide with the common advice to prevent Cryptosporidium infection is to ensure that all newborn calves ingest an adequate amount of colostrum during their first 24 h of life (Robertson et al. 2014).

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