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A Study of Oxidative Stress and Some Immunological Parameters as a Result of Cryptosporidiosis Among Animal Handlers

Sawsan Abdul Faraj Mohammed, Sunbul Jalal Shakour
Collage of Nursing, University of Kirkuk, Iraq.
sawsanabd@uokirkuk.edu.iq, sunalsaffar9@uokirkuk.edu.iq

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ABSTRACT

Cryptosporidium parvum is a common and varied intracellular apicomplexan parasite with veterinary and public health implications. C. parvum has been linked to digestive system disease in a range of host animals, including cattle, sheep, goats, and humans. Therefore, the current study aimed to evaluate oxidative stress and some immunological parameters as a result of cryptosporidiosis among animal handlers. The study included 50 people who have had experience with domestic animals (butchers and breeders). They were 10 to 50 years old. There were 43 men and 7 women. As a control, 30 seemingly healthy persons (20 males and 10 females) who had no interaction with animals and no occurrences of diarrhea in the previous two months were evaluated. A total of 120 stool and blood samples were taken from three different domestic animal species (40 cattle, 40 sheep, and 40 goats). Their contact with people involved in this study was the criterion for inclusion. Cattle 11 (27.5%) had a higher infection rate, whereas goats 6 (15%) had the lowest rate. About oxidative status, it was found that MDA levels were significantly increased ($P \leq 0.05$) and GSH and catalase levels were significantly decreased ($P \leq 0.05$) in patients with all parasitic infections compared to the control group. For cytokines, it was found that IL-4 and IL-6 levels were significantly increased ($P \leq 0.05$) in animal handlers with all parasitic infections compared to the control group. So, Cryptosporidium infection leads to increased levels of oxidative stress, cytokines, and reduced levels of antioxidant enzymes.

INTRODUCTION

Cryptosporidium, an intracellular protozoan parasite, is one of the most frequent parasitic infections causing gastrointestinal disease in humans as well as a broad spectrum of animals globally [1-2]. The protozoon parasite Cryptosporidium is the cause of the intestinal sickness known as cryptosporidiosis. It is one of the most prevalent waterborne illnesses and the main factor behind outbreaks around the world [3-6]. In general, cryptosporidiosis is spread through the fecal-oral route, and infections in humans are thought to be caused by contact with animals, manure, or polluted food and drink [7-8]. In animals, transmission happens mostly through ingestion of oocysts discharged by animals that are infected, particularly neonates in overcrowded or mixed living settings. Manure generated by livestock, particularly cattle, is a significant source of infection for both animals and humans, with an estimated global Cryptosporidium burden of 3.2X1023 oocysts each year [9]. The parasite typically spends the majority of its life inside a specific "parasitophorous vacuole," which also helps the infection avoid detection by the immune system of the host [10–11]. Cryptosporidium-induced inflammasome activation and pattern recognition receptors have also been discovered [12–13]. Despite the fact that in most animals infections are not necessarily accompanied by clinical signs and are usually believed to be a self-limiting disease [14-15], they can be fatal in severely immunocompromised people who are at risk of recurring infections [16]. Infected people exhibit a variety of clinical symptoms, including diarrhea, vomiting condition, nausea, stomach cramps, and a low-grade fever [17]. The phrase "oxidative stress" describes a shift in the steady-state oxidant to antioxidant ratio in favor of oxidants in cells. Its synthesis is brought on by an excess of reactive oxygen species (ROS). To avoid oxidative stress damage, a balance between ROS and primary antioxidant defenses is required. These defenses are mostly made up of antioxidant scavenging enzymes including superoxide dismutase, catalase, and glutathione. There are very few studies that show how ROS are involved in the pathophysiology of C. parvum infection [18–20] to study the pathogenic potential of C. parvum.

Materials & Methods

Human subjects

The study included 50 people who have had experience with domestic animals (butchers and breeders) (depending on the consent of the people participating in the current study). They were 10 to 50 years old. There were 43 men and 7 women. As a control group, 30 apparently healthy persons (20 males and 10 females) who had no contact with animals and no occurrences of diarrhea in the previous two months were studied.

Animals subjects

A total of 120 fecal samples were collected from three different domestic animal species (40 cattle, 40 sheep, and 40 goats). The interaction with study participants was a requirement for admission. The aim was to examine the aforementioned types of domestic animals to ensure they are infected with C. parvum and to link them to people infected with the same parasite to confirm the transmission of the parasite from domestic animals to both butchers and breeders.

Blood samplings

After 10-12 hours of fasting, blood samples were obtained from human subjects (those in contact with animals and healthy controls). Before centrifugation, specimens of blood were stored in EDTA tubes. The specimens were centrifuged for 10 minutes at 3500 rpm, and the serum samples were separated and stored in Eppendorf tubes. Following the completion of the sample collection processes, all specimens were processed for biochemical tests. Biob Chech, Inc provided the reagents for interleukin 1, interleukin 6, MDA, GSH, SOD, and catalase, and the ELISA Awareness-Stat Fax (300).

Parasite diagnosis

Direct fecal smears stained with modified Ziehl-Neelsen [21]. The working method is by centrifuging each stool sample after dissolving it in a 10% formalin solution at a rate of 2500 revolutions/minute for 10 minutes. The upper layer of the precipitate was removed using a pipette and a thin smear was prepared from it on a clean glass slide. Smears were stained with Carbol Fuchsin for 3–5 minutes. The slides were then immersed in methylene blue dye for one minute. The slides were then washed well with distilled water, dried in air, and then examined with a high-power microscope.

Statistical analysis

The average of three readings from each sample is included in the study results. In order to determine whether the results of the study were considered to have significant differences at the probability level (P 0.05) or not to have significant differences, a one-way analysis of variance (ANOVA) test was used [22].

Results & Discussion

In the current study, cryptosporidium infection was detected in animals that were selected for the purpose of verifying whether the same infection was found in both butchers and breeders. Therefore, table (1) displays the prevalence of cryptosporidium infection in animals. Cattle 11 (27.5%) had a higher infection rate, whereas goats 6 (15%) had the lowest rate. Out of a total of 120 animals, 26 (21.7%) were infected overall among the study animals

Table (1): Cryptosporidium among studied species of animals

Animals	No. Examined	No. positive results
Cattles	40	11 (27.5%)
Sheep	40	9(22.5%)
Goats	40	6(15%)
Total	120	26(21.7%)

In the current study, the percentage difference in cryptosporidium infection between Cattles, Sheep, and Goats may be due to a variety of factors, including the environment, the way the animals were raised, the season, the temperature, and the humidity level of the study area, the manner of treatment, the drugs used [23]. The study's three domestic animals had a 21.7% frequency of cryptosporidiosis, this rate of infection in current study was lower than the frequency of cryptosporidiosis in domestic animals recorded in the middle of Iraq (33.37%, 39.8%) [24-25]. The inclusion of diarrheal newborn calves, which have an increased incidence than older cattle, may be back to the higher infection rates observed in cattle [26-27]. In this investigation, the prevalence rate among goats was lower than that reported in Australia (85%) among diarrhoeal children [28]. In addition, in the current results, there was a difference in the rates of cryptosporidium infection in sheep and goats, as the infection in sheep was higher than in goats, and the results of the current study differed with the study of Angus et al. [29] who found that the rate of cryptosporidium infection in sheep was lower than in goats, and the reason may be due to the size and number of samples that were taken for animals in both the current study and the study above.

Table (2) shows the parasite infection rate in diarrhea patients. E. histolytica caused the highest percentage of infection (33%) while E. vermicularis caused the lowest rate of infection (4%). The overall infection rate among the investigated animals was 26(21.7%) out of a total of 120 animals. The infection rate with Cryptosporidium was 7 (14%).

Table (2): parasite types among diarrhea patients

Parasite	Type of contact with animals		Number of positive results	Percentage
	Butcher	Breeder		
Cryptosporidium	2	5	7	14%
Entamoeba histolytica	8	25	33	66%
Giardia lamblia	3	5	8	16%
Enterobius vermicularis	0	2	2	4%
Total	13	37	50	100%

The proportion of Cryptosporidium infection in this study was 14%, which was consistent with other studies conducted in different countries where the infection rate of Cryptosporidium varied (10-55.56%) [30-33]. Regarding E. histolytica infection in current study , it was lower than study of Jasim et al. [34] in Egypt, who discovered that Entamoeba spp. was present in 74.8% of cattle less than 12 months old. In the current study, 16% of butchers and breeders had Giardia lamblia infection, which was consistent with Kashash's [35] finding that the infection rate of Giardia lamblia in Baghdad was 14.6%.

Table (3) demonstrates a substantial shift in MDA and antioxidant enzyme levels among animal handlers suffering from parasite diarrhea. MDA levels were found to be significantly higher (P≤0.05) in patients with all parasite illnesses, while GSH and catalase levels were significantly lower (P≤0.05) in the control group.

Table (3): parasite types and oxidative status among diarrhea patients

Parasite	MDA (mean ± SD)	GSH (mean ± SD)	CAT (mean ± SD)
Control	1.37±0.15	0.459±0.03	1.19±0.08
Cryptosporidium	2.09±0.21*	0.284±0.025*	0.84±0.03*
E. histolytica	2.26±0.15*	0.304±0.011*	0.67±0.09*
G. lamblia	2.14±0.27*	0.371±0.014*	0.81±0.05*
E. vermicularis	1.93±0.08*	0.351±0.029*	0.74±0.02*

*: means there is a significant difference (P≤0.05) between the groups. The control group is compared with the rest of the groups for each parameter.

Additionally, Antioxidants shield both humans and animals from viral infections by preventing the scavenging of free radicals. Additionally, SOD catalyzes the lethal superoxide radical's conversion into hydrogen peroxide, a less harmful compound [36]. MDA, on the other hand, is the most common aldehyde produced as a result of lipid peroxidation and serves as a major indicator of oxidative damage to cell membranes [37]. The current investigation supports prior findings by El-Sayed and Fathy [38] and Bhagat et al. [39] that handling sick animals increased MDA levels, which in turn caused oxidative stress. Other studies that indicated a reduction in SOD activity [40] previously described oxidative damage during cryptosporidiosis. In addition, results demonstrate elevated levels of MDA and decreased GSH and catalase levels in rats injected with intestinal parasites according to Al-Kaky [41], who stated that patients with intestinal parasites have higher levels of MDA and lower levels of GSH when compared to the control group.

Table (4) demonstrates a significant shift in cytokine levels among animal handlers suffering from parasite diarrhea. IL-4 and IL-6 levels were observed to be substantially higher (P≤0.05) in patients with all parasite infections compared to the control group.

Table (4): parasite types and IL-1 and IL-6 among diarrhea patients with cryptosporidiosis

Parasite	IL-4 (pg/ml) (mean ± SD)	IL-6 (ng/L) (mean ± SD)
Control	0.389±0.138	17.938±0.671
Cryptosporidium	0.921±0.217*	26.302±2.293*
E. histolytica	0.965±0.118*	30.931±3.711*
G. lamblia	0.844±0.093*	25.527±2.418*
E. vermicularis	0.739±0.136*	22.855±1.279*

*: means there is a significant difference (P≤0.05) between the groups. The control group is compared with the rest of the groups for each parameter.

Serum levels of interleukin-4 and interleukin-6 increased significantly in animal handlers infected with intestinal parasites; This study would suggest that both intestinal parasites invade the gastrointestinal tract and infect their hosts through ingesting cysts. They bind to the intestinal mucosal surface of the duodenum in people with G. lamblia colon and trigger an immune response that results in the generation of IL-6 by T cells, dendritic cells, and mast cells [42]. An increase levels of some cytokines, such as IL-4 and IL-6, suggests the presence of inflammation [43].

Conclusions

According to the findings of the current study, infection with Cryptosporidium and other parasites of animal handlers causes higher levels of oxidative stress, certain cytokines, and decreased levels of antioxidant enzymes.

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