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Evalution the association between the infection with *Cryptosporidum spp*. and some immunological parameters

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ABSTRACT

The coccidian infection cryptosporidiosis affects humans, domestic animals, and untamed vertebrates. The primary objective of this research was to assess the prevalence of *Cryptosporidium spp*. among diverse groups of individuals residing in Al-Anbar Province. These individuals sought medical care across a range of healthcare facilities, this encompasses facilities such as Al-Ramadi Maternity and Children's Hospital, Fallujah Teaching Hospital, IDP camps, well-frequented health centers in Al-Anbar Province, and privately-operated laboratories. The data collection period encompassed the timeframe from March 2022 to February 2023. Additionally, the study aimed to investigate potential correlations between *Cryptosporidium spp*. infection and various factors, including age, gender, place of residence, and seasonal variations, as well as evalute the immunological parameters as IgM ,IgG ,IL-4 and IL-18. A total of 420 stool samples and blood serum samples were collected from individuals, including both children and adults, as well as males and females, across Al-Anbar province and private laboratories. Among the individuals tested, 60 were found to be infected with *Cryptosporidium spp.*, resulting in an infection rate of 14.3%. The research included 28 (46.7%) participants who were male and 32 (53.3%) who were female. No statistically significant variances were noted between *Cryptosporidium spp.* infection and gender. However, when considering age groups, certain groups were found to be more susceptible to infection. Notably, the highest infection rate was observed in the age group of 1 to 14 years, with 21 individuals (15.3%) affected. Regarding residency, 37 patients (15.7%) hailed from rural areas, while 23 (12.5%) came from urban areas. In terms of occupation, children exhibited a higher rate of *Cryptosporidium spp*. infection compared to individuals in other occupational categories.The study also documented the seasonality of *Cryptosporidium spp*. infections, with the highest infection rate of 31.80% occurring in April 2022, July 2022, and August 2022, corresponding to the summer season. This seasonal variation was found to be statistically significant. Furthermore, the study employed ELISA testing, which revealed that 22 serum samples (22.9%) tested positive. Specifically, IgM ELISA results showed a 10.40% positivity rate, while IgG results indicated a 12.50% positivity rate for *Cryptosporidium spp.* These results were obtained using the ELISA method.

Introduction

Cryptosporidium is a protozoan parasite that exclusively infect the intestinal tract and is a known causative agent of gastrointestinal illnesses. While initially identified in the gastric lining of mice in 1907, it wasn't associated with human infections until 1976.

It is found everywhere in the environment and across various geographical regions, with more than 40 species identified, and at least 20 of them have been associated with human infections. However, *C. hominis* and *C. parvum* are responsible for the majority of human cases. Traditionally, detecting *Cryptosporidium* oocysts relied on microscopic examination in environmental, water, food, fecal, or tissue samples. While this method

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is cost-effective and has been a fundamental diagnostic tool for many parasites, it is labor-intensive and slow[1 , 2]. Moreover, its accuracy diminishes when oocyst concentrations are low or when oocysts are damaged. Additionally, it cannot estimate past infection prevalence due to the short sporadic shedding of oocysts. Therefore, ELISA offers a superior diagnostic approach by targeting oocyst antigens and providing improved sensitivity compared to microscopy. In both humans and domestic animals, *C. parvum* parasites invade the microvillus brush border of enterocytes in the intestines. The immune system, comprising innate and adaptive components, plays a crucial role in defending against *C. parvum* and clearing infections, whether primary or secondary. During cryptosporidiosis, the immune response typically involves the activation of systemic helper T1 cells (Th1 type). Intestinal intraepithelial lymphocytes play a pivotal role in combating cryptosporidiosis by initiating control of the infection through Th1 responses and eliminating the parasites through cytotoxic T-cells[3,4] . Previously, it was suggested that interferon (IFN)-γ and other proinflammatory cytokines like interleukin (IL)-1β and tumor necrosis factor (TNF)-α could act directly on intestinal epithelial cells (IECs) to control parasite development. It is now widely acknowledged that IECs serve as crucial infection sensors by producing various proinflammatory cytokines, chemokines, and antimicrobial peptides, which may also have autocrine effects on the epithelium. Additionally, there's evidence to suggest that epithelial cells, including IECs, may generate IL-18. IL-18 plays a significant role in initiating a protective Th1 response against a broad spectrum of pathogens. Furthermore, studies indicate that IL-18 can have a distinct proinflammatory function, as it enhances the production of IL-1β, TNF-α, and IL-18 itself in macrophages and neutrophils when treated with IL-18. Previous research has also shown increased production of IL-18 by macrophages in response to direct infection, and the expression of this cytokine by epithelial cells may also change following infection[[6] 6, 7].

Materials and methods

Sample collection involved acquiring a total of 420 stool and blood serum samples from a diverse range of patients, encompassing children and adults, as well as both males and females. These samples were sourced from various healthcare facilities, , and private laboratories. The data collection spanned from March 2022 to February 2023. Stool samples, each weighing around 20 grams, were meticulously collected and placed in sanitized plastic containers with secure lids to maintain sample moisture and prevent drying out. These samples were then conveyed to the parasitology laboratory at Al-Ramadi Maternity and Children Hospital. Additionally, patients were furnished with a questionnaire to gather information about their place of residence, gender, age group, occupation, and any relevant seasonal variations for the study.

Subsequently, the collected stool samples were examined within a timeframe of no more than thirty minutes from the time of collection. Optical microscopy was utilized, employing direct smear methods for the detection of various intestinal parasites[8].

Blood serum samples Blood samples, approximately 5 milliliters in volume, were collected from individuals randomly selected among those from whom stool samples were obtained. These blood samples were drawn using plastic medical syringes. The collected blood was then transferred into clean plastic test tubes and allowed to sit for a period of 15 minutes to facilitate the natural clotting process. After clotting had occurred, the blood samples were subjected to centrifugation at a speed of 4000 revolutions per minute (rpm) for a duration of 10 minutes. This centrifugation process resulted in the separation of the blood serum from other blood components. The obtained blood serum was then carefully transferred into Eppendorf tubes, to preserve the blood serum for subsequent immunological tests as outlined in the study, the Eppendorf tubes containing the serum were immediately stored at a temperature of -20°C [9].

Direct Wet Method The examination of stool samples involved the use of direct smear methods. Clean glass slides were employed for this purpose. A small drop of either normal saline solution (0.9%) or iodine stain was applied to the glass slide. This solution was mixed thoroughly with a small portion of the feces sample

using a wooden stick. Subsequently, coverslips were placed on the slides, and the prepared samples were examined under a microscope at magnifications of 40X and 100X [10].

Ziehl-Neelsen Staining To prepare the sample for examination, a small amount of feces, approximately the size of a matchstick head, was mixed with a drop of distilled water on a glass slide. This mixture was spread evenly across the slide and allowed to air-dry for about 10 minutes. To fix the smear, it was immersed in 100% absolute methanol for 5 minutes and then left to dry.The next step involved immersing the slide in carbol-fuchsin for a duration of 3 minutes. Afterward, the slide was thoroughly rinsed with tap water to remove excess staining. To decolorize the red color, acidic alcohol was applied for 30 seconds, followed by another rinse with tap water. Subsequently, the slide was exposed to methylene blue for a period of 2 minutes and then washed again with tap water before being left to dry. Finally, the prepared slide was examined under a microscope using an oil immersion objective lens with a magnification of 100x to check for the presence of oocysts[11].

Measurement of antibodies (IgG and IgM) by ELISA We followed the protocol as per the instructions provided by the supplier (Sunlong, China) , we tested these antibodies for diagnosis of infection.

Estimate levels of Interleukins(IL18,IL4) The concentrations of these interleukins in the blood serum samples were determined using the ELISA technique, following the manufacturer's instructions.

Statistical analysis The collected data were subjected to statistical analysis, utilizing the Chi-square test for categorical variables and ANOVA (Analysis of Variance) to discern differences in means. The analysis was performed using the SPSS software program [5].

Results and discussion

In our present study, we have put forward a thorough strategy that incorporates traditional immunological and to identify Cryptosporidium *spp*. in fecal samples and blood serum gathered from a diverse demographic, including both male and female children and adults. These samples were sourced from a range of healthcare institutions. The study was conducted over the course of one year, spanning from March 2022 to February 2023.Table (1) in our study provides an overview of the overall prevalence of *Cryptosporidium spp*. infection in Al-Anbar province. Among the individuals tested, 60 were found to be infected with a corresponding infection rate of 14.3%. In contrast, 360 individuals tested negative for *Cryptosporidium spp*., accounting for an 85.7% non-infection rate based on microscopic examination.

Table 1: The occurrence of *Cryptosporidium spp*. in the Al-Anbar province.

Number of samples	420
Positive (%)	60(14.3%)
Negative (%)	360(85.7%)

The infection rate of the parasite observed in the current study is comparatively lower than the rates reported in numerous earlier studies conducted in Iraq, as indicated in previous research in Wasit province was 33.83% , in diffrent areas in Iraq was 18.3% and in Basra Province was 23.8% . The infection rate of the parasite documented in the current study is higher than the rate reported in certain other studies conducted in Iraq, as noted in previous research in Erbil City-Kurdistan region, Iraq was 14% , in Mid-Euphrates area was 8.35% and in Basra was 5%. The disparity in environmental and climatic conditions across the study areas, as well as the presence or absence of specific animal reservoir hosts for various parasites, can significantly influence the infection rates. Additionally, factors such as the sample size, examination methods employed, and the comprehensive assessment of both pathogenic and non-pathogenic parasites play crucial roles in determining infection percentages [1,8, 12, 13, ,14 ,15,16].

Regarding gender distribution, the research included 28 (46.7%) male participants and 32 (53.3%) female participants, as reported in table (2). These results correspond with earlier studies conducted by in Wasit province, Iraq, where the infection rate among females was 41.2% and among males was 4.8%, showing no significant disparities. Similarly, the study conducted in China demonstrated an infection rate of 48.8% among males and 51.2% among females. The variation in infection rates between genders could

potentially be attributed to the different types of work undertaken by females and males. Females may experience increased exposure to risk factors when handling domesticated animals, coupled with potential gaps in awareness regarding personal hygiene. Furthermore, the higher infection rate among females might be linked to their increased exposure to untreated water sources compared to males. Additionally, excessive contact with potential sources of infection, such as handling food waste, household waste, and engaging in gardening activities, could contribute to this discrepancy. The findings from our study strongly suggest that certain age groups are more susceptible to infection. Table (2) clearly illustrates this pattern, with the highest infection rate observed in the age group of 1 to 14 years, encompassing children and primary school students. Conversely, the lowest infection rate was noted in the age group of 45 to 59 years, with a rate of 7 individuals (17.5%), and this difference did not reach statistical significance with a P value of ($P \leq 0.05$). These results align well with the outcomes reported in previous studies conducted by in Taiz District, Similar patterns of higher infection rates among specific age groups have been observed in other regions, such as Yemen, where the highest infection rate was reported among the age group of 2 to 6 years, reaching 40.3%. Additionally, in a study conducted in North Shewa Zone, Ethiopia, a higher rate of infection (8.5%) was found among individuals who had close contact with animals. However, it's worth noting that there are contrasting findings from studies like the one conducted in the Venda region of Limpopo Province, South Africa, which explored the prevalence and genotype distribution among school children and hospital patients. In this study, the rate of infection was notably high among individuals in the age group of 50 to 59 years old, with a prevalence of (50.0%). These disparities in results could potentially be attributed to a higher susceptibility to *Cryptosporidium spp.* infections among individuals who may lack awareness regarding safe food and water practices .Children in certain areas exhibit behaviors that increase their risk of *Cryptosporidium* infection. These behaviors include not practicing proper hand hygiene before eating, engaging in activities in soil and sewage-contaminated water, having frequent exposure to fecal-oral transmission routes, and consuming contaminated food or water. Additionally, some children residing in villages heavily rely on untreated well water for drinking, especially during the summer when piped water is scarce. This untreated well water, stored during the winter, provides an environment conducive for *Cryptosporidium* oocysts and other pathogens to persist. Furthermore, these children often consume food outside their homes and may come into contact with the parasite during manual labor or in dusty environments[2,3, 6,12, 16 ,17,18]. Based on the location of residence, the study identified 37 patients (15.7%) from rural areas and 23 patients (12.5%) from urban areas, as indicated in Table 2. These findings are in line with the results of previous research conducted in various countries, such as a study in Wasit province, Iraq. However, they differ from the findings of a study in Iran, where the infection rate in urban areas (66.7%) was higher than in rural areas (33.3%) .The variation in infection rates between rural and urban areas can be attributed to several factors. In rural settings, there may be a higher level of environmental exposure to potential sources of infection, including contaminated water sources, the presence of farm animals, and grazing by cattle and sheep. Particularly during the rainy season, heavy rainfall can contribute to the spread of *Cryptosporidium* oocysts from infected animal feces, leading to contamination of drinking water supplies. To mitigate the risk of *Cryptosporidium* infection, it is crucial to implement preventive measures such as the use of water purification systems and restrictions on grazing farm animals near drinking water sources. These measures are essential for safeguarding the quality of drinking water and reducing the transmission of *Cryptosporidium*. The study's findings revealed that the percentage of *Cryptosporidium spp.* infection varies based on occupation, with children experiencing a higher infection rate compared to individuals in other occupations. Specifically, the modified acid-fast staining method identified an infection rate of 18.4% among preschool-aged children, as detailed in Table 2. However, there was no statistically significant difference in the incidence of *Cryptosporidium spp.* infection based on occupation[16, 19,20].

These results align with previous research conducted in the West Bank, Palestine, which reported an infection rate of 11.6% among children, as well as another study in Bethlehem, West Bank, where a prevalence rate of 13.5% was observed among children with diarrhea[21][22]. However, they contrast with a study in Qatar, where a higher infection rate was documented among elementary school students (7.1%) compared to younger children (1%). This discrepancy may be attributed to the challenging living conditions faced by children in certain areas, coupled with factors like a lack of self-awareness, inadequate personal hygiene practices, and cleanliness during this critical stage of development. These conditions place children at a higher risk of contracting Cryptosporidiosis[23].The study also analyzed the seasonal variation in *Cryptosporidium spp*. infections, and the results are summarized in Figure 1. Notably, the highest infection rates, reaching 31.80%, were recorded in April 2022, July 2022, and August 2022, which coincide with the summer season. This seasonal variation was statistically significant (P<0.05), highlighting the influence of seasonal factors on *Cryptosporidium* infection rates.

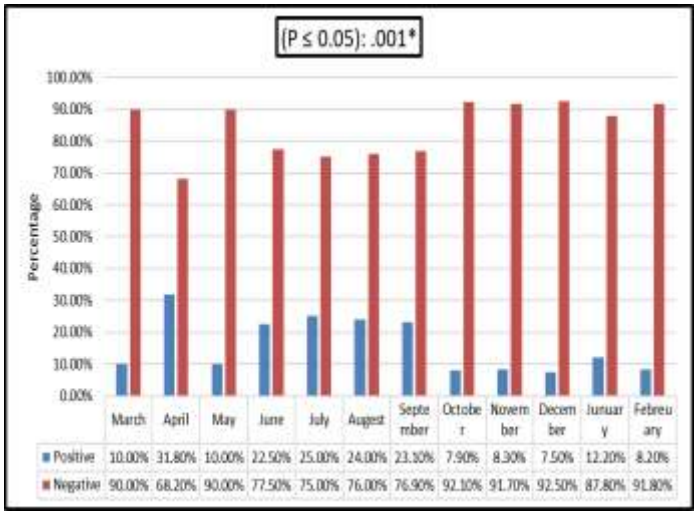

Figure 1: illustrates the prevalence of *Cryptosporidium spp.* in relation to seasonal variations

Certain studies yielded distinct findings. For instance, in the New York City Watershed, as observed in, there was a higher prevalence of *C. parvum* during the summer (26%) compared to the winter (11%). However, our findings contrasted with several other studies, including the research by[24] in Jiangsu Province, China, which reported a higher infection rate of 29.1% in the Autumn season.This differences in results The variation in *Cryptosporidium* infection rates throughout the year could be attributed to several factors. In months with the highest infection rates (April, July, and August), relatively warm temperatures and various epidemiological factors may come into play. These factors may include minor differences in temperature and humidity, as well as increased water availability, all of which create favorable conditions for the contamination of drinking water supplies with animal feces and sewage. The warmer weather can promote the survival and transmission of *Cryptosporidium* oocysts.Conversely, the low infection rates observed in winter months (January and February) could be attributed to the severe drop in temperatures during this season, often reaching or falling below freezing (0°C or less). Such extreme cold temperatures can have a detrimental impact on the viability and infectivity of *Cryptosporidium* oocysts, reducing their ability to cause infections. It's worth noting that climate change can also influence the incidence of infections like rotavirus. Other studies have found a higher incidence of rotavirus gastroenteritis occurring between December and April. Additionally, during the summer months, increased recreational activities such as swimming, travel, and visits to parks can contribute to injury and potentially play a role in the transmission of infections[23, 25].

Table 2: Statistical information describing the study's participant group.

Age group (year)	Microscopic diagnosis	positive	negative	Total
	1<-14	21(15.3%)	116 (84.7%)	137
	15-29	10 (11.0%)	81(89.0%)	91
	30-44	13 (13.4%)	84(86.6%)	97
	45-59	7 (17.5%)	33(82.5%)	40
	60-<74	9 (16.4%)	46 (83.6%)	55
	Total	60 (14.3%)	360 (85.7%)	420 (100%)
	P value (P ≤ 0.05).	.823NS	.817NS	.706NS
Occupation	Microscopic diagnosis	positive	negative	Total
	Child	18 (18.4%)	80 (81.6%)	98
	Primary school	2 (6.9%)	27 (93.1%)	29
	Student	7 (12.7%)	48 (87.3%)	55
	Officer	15 (18.1%)	68 (81.9%)	83

	Housewife	**10 (11.4%)**	**78 (88.6%)**	**88**
	Wage earner	**1(2.7%)**	**36 (97.3%)**	**37**
	Retired	**7 (23.3%)**	**23 (76.7%)**	**30**
	Total	**60 (14.3%)**	**360 (85.7%)**	**420 (100%)**
		.110NS	**.062NS**	**.468NS**
Gender	**Microscopic diagnosis**	**positive**	**negative**	**Total**
	Male	**28 (46.7%)**	**155 (43.1%)**	**183 (43.6%)**
	Female	**32 (53.3%)**	**205 (56.9%)**	**237(56.4%)**
	Total	**60 (14.3%)**	**360 (85.7%)**	**420 (100%)**
	P value (P ≤ 0.05).	**.601NS**	**.703NS**	**.602NS**
	ODD (CI95%)	**1.157 (.669 – 2.003)**		
Residency	**Microscopic diagnosis**	**positive**	**negative**	**Total**
	Rural	**37(15.7%)**	**199(84.3%)**	**236**
	Urban	**23(12.5%)**	**161(87.5%)**	**184**
	Total	**60 (14.3%)**	**360 (85.7%)**	**420 (100%)**
	P value (P ≤ 0.05).	**.356NS**	**.434NS**	**.353NS**
	ODD (CI95%)	**1.302 (.743 - 2.279)**		
NS: Non-significant difference at the 0.05 level by chi-square test				

Ninety-six blood samples were collected from individuals of varying ages and genders who were suspected of having a particular condition(who persons suffering from intestinal symptoms . These samples were subjected to ELISA analysis. The results revealed that only 22 of the serum samples, which accounts for approximately 22.9% of the total, tested positive for the applied test. Furthermore, when assessing the results specifically for IgM using ELISA, it was found that approximately 10.40% of the samples were positive, while approximately 12.50% of the samples tested positive for IgG in the context of *Cryptosporidium spp*., as illustrated in Figure 2. Moreover, an examination was conducted to explore the correlation between IgG concentration and age groups as well as gender. The analysis revealed that the highest IgG concentration was observed in males within the age groups of 1-14, 15-29, and 30-44 years. Additionally, statistically significant differences in IgG concentration were observed between the 30-44 age group and the 60-74 age group, as detailed in Table 3.

Figure 2: The rates of IgG and IgM by ELISA test

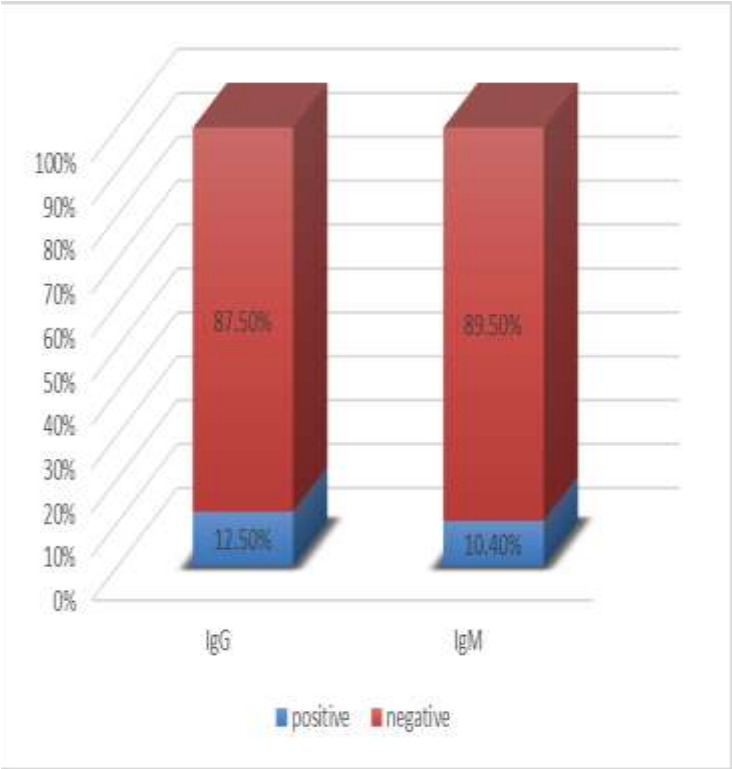

Table 3: Correlation between IgG and age groups and gender

Patients		**Gender**			**Age group**
		Male	**Female**	**Total**	
IgG concentration	**Mean**	**1.41304**	**.38009**	**1.07885**	**1<-14**
	Std. Deviation	**.505589**	**.057730**	**.350261**	
	P value (p ≤ 0.05)	**.171 NS**			
IgG concentration	**Mean**	**.63100**	**.60300**	**.61175**	**15-29**
	Std. Deviation	**.047109**	**.128718**	**.088261**	
	P value (p ≤ 0.05)	**.889 NS**			
IgG concentration	**Mean**	**1.31800**	**.38719**	**.67048**	**30-44**
	Std. Deviation	**.689987**	**.049258**	**.221372**	
	P value (p ≤ 0.05)	**.050***			
IgG concentration	**Mean**	**.42733**	**.066884**	**.62825**	**45-59**
	Std. Deviation	**.69522**	**.220600**	**.167245**	
	P value (p ≤ 0.05)	**.514 NS**			
IgG concentration	**Mean**	**.52767**	**.21460**	**.38536**	**60-<74**
	Std. Deviation	**.075719**	**.053618**	**.067204**	
	P value (p ≤	**.010***			

	0.05)		
* Significant difference under p ≤ 0.05 by One way – ANOVA NS: Non-significant difference			

Additionally, we conducted an analysis to examine the relationship between IgG concentration and age groups and gender, as presented in Table 4. Interestingly, we observed a slightly elevated IgG concentration in two specific age groups, namely, those aged 1-14 and 60-74. Notably, among males, we identified significant differences in IgG concentration when compared to all age groups.

Table 4: Association between IgM levels and age categories and gender.

Patients		IgM concentration		P value (p ≤ 0.05)
Age group	Gender	Mean	Std. Deviation	
1<-14	Male	.82239	.261547	.937 NS
	Female	.86409	.522556	
	Total	.83588	.240068	
15-29	Male	.50160	.123325	.331 NS
	Female	.38900	.052033	
	Total	.42419	.051865	
30-44	Male	.34614	.084680	.718 NS
	Female	.42688	.139427	
	Total	.40230	.099390	
45-59	Male	.24600	.060086	.260 NS
	Female	.58389	.157337	
	Total	.49942	.124952	
60-<74	Male	.76250	.222322	.121 NS
	Female	.33080	.060966	
	Total	.56627	.137027	
NS: Non- Significant difference under p ≤ 0.05 by One way – ANOVA				

The overall point prevalence of IgG antibodies to *Cryptosporidium* in the surveyed communities of Al-Anbar province was found to be 12.50%. It's important to note that the presence of IgG antibodies to *Cryptosporidium* doesn't necessarily indicate an active infection but suggests that individuals in the surveyed communities had been infected with *Cryptosporidium* at some point in their lives.

This finding implies that a majority of individuals in these communities have experienced *Cryptosporidium* infection at least once. Interestingly, some other studies have reported a higher prevalence of *Cryptosporidium* antibodies than initially expected. For instance, 86% of randomly sampled blood donors in Australia, 17% of hospital personnel (who were presumed to have minimal exposure) in a U.S. hospital, and 26% of randomly selected sera from two diagnostic laboratories in Great Britain[3,26] tested positive for *Cryptosporidium* antibodies. It's possible that the specific IgG antibody response may persist in individuals for varying durations after the initial infection.

For example, in one study, the IgG response disappeared within 12 months in four individuals[27][38].

According to Table 5, the concentration of IL-4 varied across different age groups. Among patients, the highest IL-4 concentration was observed in the age group of 45-59, with an average of 6.246 ± 1.688 pg/mL, while the lowest concentration was found in the age group of 60-<74, with an average of 3.043 ± 0.464 pg/mL. Conversely, in the control group, the highest IL-4 concentration was noted in the age group of 15-29, at a rate of 24.492 ± 10.972 pg/mL, while the lowest concentration was recorded in the age group of 60-<74, at 7.033 ± 2.098 pg/mL.. However, significant differences were found between patients and controls in the age groups of 1<-14, 15-29, 30-44, and 60-<74.

Table 5: The concentration of IL-4 in patients according to age groups

Age group		1<-14	15-29	30-44	45-59	60-<74	Total	P value (p ≤ 0.05)
Concentration of IL-4 Mean ± Std. Deviation	Patient	4.196 ± .808	4.879 ± 2.649	3.558 ± .490	6.246 ± 1.688	3.043 ± .464	4.233 ± 1.628	.060 NS
	Control	24.183 ± 11.865	24.492 ± 10.972	23.206 ± 11.680	7.033 ± 2.098	9.233 ± 1.530	18.039 ± 3.136	.188 NS
P value(p ≤ 0.05)		.000 *	.007*	.013S	.724 NS	.001*		
*Highly significant difference under p ≤ 0.05 by One way – ANOVA S: significant difference. NS: Non- significant difference								

IL-4, a glycoprotein, originates from CD4+ T lymphocytes, mast cells, and basophils. Its primary function involves prompting the differentiation of CD4+ T cells into Th2 cells while inhibiting the formation of Th1 cells. Furthermore, it serves as a growth factor for B cells, T cells, and mast cells. IL-4 is among the most potent cytokines with diverse functions, often acting in opposition to IFN-γ. Its wide array of expressed

receptors allows it to primarily affect certain cell types. It plays a pivotal role in the hormonal immune response, encouraging the growth and differentiation of B cells. Additionally, IL-4 stimulates the expression of MHC II molecules and supports isotype switching in mice towards IgG1 and IgE, while inhibiting switching to IgG2a, IgG2b, and IgG3. Furthermore, IL-4 serves as a growth factor for mast cells and exerts significant regulatory control over allergic reactions, including processes like the release of mast cell granules[3, 6]. Based on the findings presented in Table 6, our research observed that the highest concentration of IL-18 in patients was noted in the age group of 30-44, registering at a rate of 1.887 ± 0.117 pg/mL. Conversely, the lowest IL-18 concentration in patients was observed in the age group of 60-<74, with an average of 1.257 ± 0.262 pg/mL. It's worth noting that there were no significant differences in IL-18 concentrations among patients across all age groups, and the same non-significant pattern was observed in the control group across age groups. However, a notable finding was the presence of a significant difference between patients and controls within the 30-44 age group. This variation in IL-18 levels across age groups could be attributed to an enhanced immune response in the 30-44 age group or potentially to prior exposure to *Cryptosporidium spp.*

Table 6: The concentration of IL-18 in patients according to age groups

Age group		1<-14	15-29	30-44	45-59	60-<74	Total	P value (p ≤ 0.05)
Concentration of IL-18 Mean ± Std. Deviation	Patient	1.540 ± .705	1.354 ± .205	1.887 ± .117	1.703 ± .898	1.257 ± .262	1.557 ± .125	.633 ^{NS}
	Control	4.096 ± 2.659	1.964 ± .539	.925 ± .032	.796 ± .029	.746 ± .038	1.855 ± .696	.474^{NS}
P value(p ≤ 0.05)		.103 ^{NS}	.251 ^{NS}	.001*	.370 ^{NS}	.163 ^{NS}		

*** Significant difference under p ≤ 0.05 by One way – ANOVA NS: Non- significant difference**

Over the last decade, it has become increasingly clear that the gastrointestinal epithelium actively contributes to the host's innate immunity by producing various soluble mediators, including the immunomodulatory cytokine known as IL-18. IL-18, belonging to the IL-1 superfamily, is produced by cells like macrophages. It functions by binding to the interleukin-18 receptor, and when combined with IL-12, it triggers cell-mediated immunity in response to infections caused by microbial products such as lipopolysaccharide (LPS)[28]. When activated by IL-18, both natural killer (NK) cells and certain T cells release a noteworthy cytokine known as interferon-gamma (IFN-γ) or type II interferon. This cytokine plays a crucial role in the activation of macrophages and other immune cells. In addition to its typical physiological functions, IL-18 has the capacity to trigger intense inflammatory responses, implying its potential involvement in specific inflammatory conditions. Interestingly, while this cytokine is linked to the pathology of various diseases, it has been found that IL-18 produced by intestinal epithelial cells (IECs) plays a crucial role in maintaining the integrity of the intestinal lining during episodes of inflammation. Studies have demonstrated that IL-18 can inhibit the reproduction of parasites in human intestinal cell lines. Furthermore, this cytokine enhances the production of a β-defensin by IECs, a protein known to neutralize *C. parvum* sporozoites. However, it's important to note that the role of IL-18 in the innate immune response against cryptosporidia has not been thoroughly investigated [29, 30].

Conclusion

Our research outcomes offer important insights into the prevalence of *Cryptosporidium spp.* within Al-Anbar province. We did not observe significant differences in infection rates among various age groups among *Cryptosporidium*-infected patients. However, we did identify a higher infection rate in rural areas compared to urban areas. Furthermore, with regard to seasonal variations, we found significantly elevated infection rates in April, July, and August. It's worth noting that the concentration of antibodies (including

IgG, IgM, and interleukins) appeared to be influenced by the age group of individuals.

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Nil

Conflict of Interest

There are no conflicts of interest.

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تقييم العلاقة بين الاصابة بطفيلي البويغات الخبيئة وبعض المؤشرات المناعية

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الخلاصة

تؤثر عدوى الكوكسيديا خفيات الأبواغ على البشر والحيوانات الأليفة والفقاريات الجامحة. كان الهدف الأساسي من هذا البحث هو تقييم مدى انتشارطفيلي البويغات الخبيئة بين مجموعات متنوعة من الأفراد المقيمين في محافظة الأنبار. سعى هؤلاء الأفراد للحصول على الرعاية الطبية عبر مجموعة من مرافق الرعاية الصحية، وهذا يشمل مرافق مثل مستشفى الرمادي للنسائية والأطفال، ومستشفى الفلوجة التعليمي، ومخيمات النازحين، والمراكز الصحية ذات التردد الجيد في محافظة الأنبار، والمختبرات التي يديرها القطاع الخاص. شملت فترة جمع البيانات الإطار الزمني من مارس 2022 إلى فبراير 2023. بالإضافة إلى ذلك، هدفت الدراسة إلى دراسة الارتباطات المحتملة بين طفيلي البويغات الخبيئة و العدوى والعوامل المختلفة، بما في ذلك العمر والجنس ومكان الإقامة والتغيرات الموسمية، وكذلك تقييم المعايير المناعية مثل IgM وIgG وIL-4 وIL-18. تم جمع حوالي 420 عينة براز وعينة مصل دم من الأفراد، بما في ذلك الأطفال والبالغين، وكذلك الذكور والإناث، من جميع أنحاء محافظة الأنبار والمختبرات الخاصة. ومن بين الأفراد الذين تم اختبارهم، وجد أن 60 شخصًا مصابون بطفيلي البويغات الخبيئة .، مما أدى إلى معدل إصابة يصل إلى 14.3%. شمل البحث 28 (46.7%) مريضا من الذكور و32 (53.3%) من الإناث. لم يلاحظ أي فروق ذات دلالة إحصائية بين البويغات الخبيئة والجنس. ومع ذلك، عند النظر في الفئات العمرية، وجد أن بعض الفئات أكثر عرضة للإصابة بالعدوى. والجدير بالذكر أن أعلى معدل إصابة لوحظ في الفئة العمرية من 1 إلى 14 سنة، حيث أصيب 21 فرداً (15.3%). وفيما يتعلق بالإقامة، جاء 37 مريضا (15.7%) من المناطق الريفية، في حين جاء 23 (12.5%) من المناطق الحضرية. من حيث المهنة، أظهر الأطفال معدل أعلى في الأصابة بالبويغات الخبيئة. مقارنة بالأفراد في الفئات المهنية الأخرى. كما وثقت الدراسة التباين الموسمي لأنتشار الطفيلي وتحدث أعلى نسبة إصابة بنسبة 31.80% في أبريل 2022، ويوليو 2022، وأغسطس 2022، الموافق لموسم الصيف. وقد وجد أن هذا الاختلاف الموسمي ذو دلالة إحصائية. علاوة على ذلك، استخدمت الدراسة اختبار ELISA، الذي كشف أن 22 عينة مصل (22.9٪) كانت إيجابية. وعلى وجه التحديد، أظهرت نتائج IgM ELISA نسبة إيجابية 10.40%، في حين أشارت نتائج IgG إلى نسبة إيجابية 12.50% لطفيلي البويغات الخبيئة تم الحصول على هذه النتائج باستخدام طريقة ELISA.

الكلمات المفتاحية : *البويغات الخبيئة، معايير مناعية، محافظة الأنبار.*