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RESEARCH ARTICLE

An Epidemiological and Therapeutic Study of the *Cryptosporidium parvum* Parasite in Some Farm Animals in Anbar Province /Iraq

Suad Shallal Shahatha

College of Science- University of Anbar Department of Biology/Iraq.

Abstract

This study was conducted to investigate of *Cryptosporidium parvum* parasite in some farm animals in Anbar Province by examining 564 feces samples (148 sheep, 144 cows, 132 goats, and 140 chickens). The results showed that the infection rates of the examined animals were 35.8 %, 41.6 %, 34.8 % and 25.7 % respectively. There were no significant differences ($p \geq 0.05$) between the sexes in infection. The infection rate in small age's groups was 46.5% while the large age's groups 21.4 %. Significant differences were observed ($p \geq 0.05$) between infection rates in rural areas and urban areas (44.9 %, 21.1) % respectively. The study also involved an effect of a water extract of the *Zygophyllum fapago* plant (also locally known as Chicken choking plant) on *Cryptosporidium parvum* parasite growth. As part of this study, a concentration of between 0.05-5 mg/ml was used. The application of these concentrations led to an inhibitory effect on parasite growth - an application of relatively higher concentrations caused greater effects in times of growth between 1-5 days.

Keywords: *Epidemiological, Cryptosporidium parvum, Animals, Extract, Anbar.*

Introduction

The *Cryptosporidium parvum* parasite is a protozoan parasite that causes cryptosporidiosis, a common disease among humans and animals [1]. A molecular study in Iraq proved for the first time that this parasite is a zoonosis parasite between snakes and humans [2]. The infection is transmitted to humans and animals through water and food contaminated with Oocysts [3, 4], or through air and blood [5]. The parasite has about 79 species [6] and affects many hosts such as birds, rabbits, fish, reptiles and Cattle [2], this parasite has a strong effect and causes severe diarrhea, especially in children less than 5 years of age and animals with small ages [7].

This parasite is one of the most virulent parasites possessing toxin-producing genes [8].The infected host subtracts large numbers of Oocysts with faces, increasing the incidence of infection in humans and different animals [9].There is no effective and definitive treatment for this disease, as more than 95 compounds were used, including paramomycin, Azithromycin, Metronidazole, Avermacin and Nitrazoxanide. However, the results were not positive and did not kill the parasite [10, 11].

So researchers sought natural alternatives such as plant extracts and medicinal herbs on pathogens such as bacteria, parasites and fungi where plants possess a natural reservoir of active chemical compounds that play an important role in inhibiting the growth of microorganisms, such as alkaloids, tannins, volatile oils, Flavonoids, glycosides sterols and phenols [12,13]. *Zygophyllum Papago* plant belongs to the family of Zygophyllaceae, and locally called Chicken choking plant or Abu Twice. It is an Annual Herb with a height of between (30- 60cm) with an ascending stem, and its leaves are bifoliolate, fleshy, obovate, and its flowers are white-cream [14] (Figure 1).

It spreads in alluvial, sandy clay soil and sunny places, blooms from early April to late July. The plant contains alkaloids; the most important is the Harmelan tripartite soap compounds, and many other glycosides, phenols and fatty acids. Plant extracts are effective against bacteria, fungus and parasites. The local population has used it as an antimicrobial and anthelmintic drug and research indicates the effectiveness of the glycosides as an anticancer. Research has also demonstrated the ability of plants to

absorb harmful mineral elements from soils contaminated with these elements [15]. This study was conducted due to the paucity of studies in Anbar province on the prevalence of this parasite in farm animals, as it is a parasite causing serious diseases that lead to many health problems and economic losses and also to find effective and safe treatment and elimination using effective plant extracts. This study is the first in the country which uses *Zygophyllum fapago* plant to influence this parasite.

Figure 1: The *Zygophyllum fapago* plant

Materials and Methods

Collection and Examination of Samples

A total of 564 feces samples were collected from some farm animal species in Anbar province at different ages (1month -5 years) and from both sexes (148 sheep, 144 cows, 132 goats, and 140 chickens). Samples were placed in plastic bottles and added to 10 mL of distilled water to prepare a suspension, which was filtered with a piece of gauze in clean bottles and 10 ml of potassium dichromate 2.5% was added as a preservative for parasite Oocysts.

The examination was performed using the modified Ziel-Neelson stain method, according to Garcia and Procop [16] where 10 ml was taken from the suspension and was centrifuged for 2500 cycles/ minute for 5 minutes. This process was repeated until the solution became clear and free of potassium dichromate. Light swabs were prepared from the sediment on slides and the smears were fixed at 60 °C for 10 minutes in the drying oven and were painted with Carbol Fuchsine for 5 minutes, then they were washed with distilled water and immersed in the dye remover for a one minute and washed with distilled water, dried with air and the slides were submerged with anti-Methylene blue dye for one minute and washed, and dried with air and examined on (40 x) and (100 x) by microscope.

Preparation of Aquatic Extract of Plant

The *Zygophyllum fapago* plants were collected from Al-Sufiya area in Al-Ramadi city for the period from early April to late July 2017, which was diagnosed in Al-Anbar University Herbarium. The plant was washed with distilled water and dried on the filtration papers and then crushed to prepare the water extract according to Pavela [17] by mixing 40 g of the plant powder with 160 ml of distilled and sterile water in a ratio of 4: 1 using the Blender.

The crush was inside a snow bath to ensure that the active compounds are not harmed by high temperature. Then, the product was stirred for 60 minutes using the electric magnetic motor Stirrer and left for 1 day at 4°C. After that, the mixture was filtrated using filtration papers to get rid of the non-crushed parts and then the plant extract was dried using Lyophilizer and the powder was kept after drying in glass bottles at -10°c until use. The standard concentration was prepared according to Riose *et al.,* [18] method by taking 2g of raw extract and 20

mL of distilled water, thus the standard concentration is 100 mg / ml. Then it was sterilized with membrane filters to prevent the passage of bacteria thus obtaining the stock solution and the preparation of the concentrations used in this study.

Effect of Different Concentrations of Aquatic Extract of Plant on Parasite Growth

The concentrations 0.05, 0.5, 1.5, 2, 5 and 5 mg/ml from the aquatic extract solution (stock) were added separately to the glass bottles containing the prepared culture media. These bottles were vaccinated with developed parasites in Maconky agar with the untreated bottles with three (control) replicates were incubated at 26°C for five days. The number of parasites and their activity were calculated after (1, 2, 3, 4, 5) days.

Chemical Detection of Bioactive Compounds in Plants (19)

Detection of Glycosides

5 ml of Benedict reagent was added to 1 ml of extract in a test tube and within a water bath heated to 100°C. After 5 minutes, the tube was cooled, and the presence of a red deposit indicated the existence of glycosides.

Detection of Flavonoids

A 10 ml solution of 50% ethanol was added to 10 ml of 50% potassium hydroxide and mixed in an equal amount; the presence of a yellow color indicated the existence of flavonoids.

Detection of Phenols

1 ml of extract was added to 1 ml of 1% ferric chloride solution; the surfacing of a green or green-like shade indicated the presence of phenols.

Detection of Alkaloids

10 grams of plant extract was boiled with 50 ml of distilled water containing 4% hydrochloric acid (HCL). The solution was cooled and filtered. Following this, 0.5 ml of leachate was tested in a watch glass with 0.5 ml of Meyer reagent; the emergence of a white deposition confirmed the presence of alkaloids.

Detection of Terpenes

A mixture of the following chemical materials was prepared: 1 gram of plant extract was dissolved in 2 ml of chloroform, followed by a drop of anhydrous acetic acid and a drop of concentrated sulfuric acid. The presence of brown sediment indicated the existence of terpenes.

Detection of the Saponins

3 ml of mercuric chloride solution (1%) was added to 5 ml of the extract; the appearance of white sediment indicated the presence of the saponins.

Detection of Tannins

A few drops of lead acetate solution (1%) were added to 5 ml of the plant extract; the emergence of white gelatin deposits confirmed the presence of tannins.

Detection of Resins

50 ml of ethyl alcohol (95%) was added to 5 grams of plant extract and heated in a water bath. The solution was then filtered and combined with 100 ml of distilled water containing 4% of HCL. The appearance of turbidity indicated the presence of resins.

Statistical Analysis

The results were analyzed using Chi-square and compared using the Least Significant Difference (L.S.D.) and the SAS [20].

Results and Discussion

The results showed that the total infection rate with *Cryptosporidium parvum* was 34.5% in the animal species examined in Anbar Province, with infection rates (35.8, 41.6, 34.8 and 25.7 %) for each of the cattle, sheep, goats and chickens respectively, (Table 1). This is consistent with what was found by [21] and [22]. The high rate of infection in Anbar Province is attributed to the lack of clean water, food and non-sterilization of drinking water, especially due to the bad conditions in previous years, which led to the outbreak of many epidemic diseases.

The main factor of the parasite transmission and infection is water and food contamination with Oocysts of parasite from the feces of other infected animals which were spread in the rivers, ponds and water ways and on the herbs and vegetables consumed by animals as well as the spread of dogs, rodents and insects, which are a source of infection and the spread of the disease.

Table 1: Number of samples examined and percentages of infection with *C. parvum* in examined animals

Animals	Numbers of examined samples	Numbers of infected samples	%
Sheep	148	53	35.8
Cows	144	60	*41.6
Goat	132	46	34.8
Chicken	140	36	25.7
Total	564	195	34.5
Significant variations			0.001

* = significant

The Oocysts of parasite are spherical, ovoid, and light red, the spores are black and the background is blue and with a size of(4.2 - 5.1) micrometers and surrounded by a transparent halo (figure 2).This is an evidence that Oocysts belong to the *Cryptosporidium parvum* species and this is consistent with the study conducted by De Jong [23].The statistical analysis did not show a significant difference at ($p \leq 0.05$) for the effect of sex on the infection, where the ratio was 48.2% for males and 51.7% for females (Table 2), this corresponds to the study by [24].

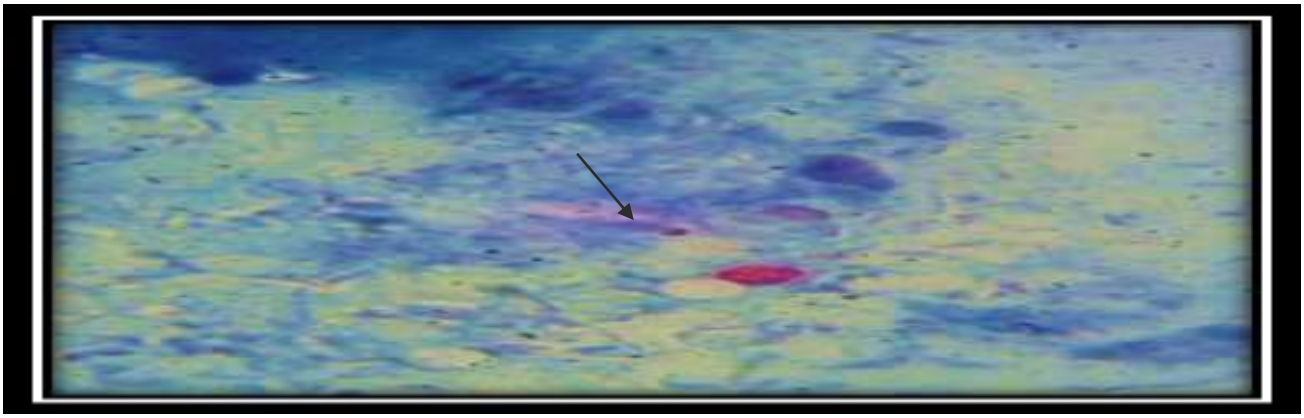

Figure 2: *Cryptosporidium parvum* Oocyst in Animal fecal Samples dyed with modified Ziel-Nelson method (40x)

The reason is that both sexes were exposed to the same environmental conditions and to similar breeding conditions in the farms and fields of livestock and to the same sources of pollution, as there is no male and female animals specified factor increases the readiness of the animal to accept the injury or contribute to resistance, In the case of isolation of males from females and in different environmental conditions and education, this led to different rates of infection, which is also confirmed by [25]. Small ages (1 month - 2 years) were 46.5% higher than those of large ages (3-5) years 21.4% with a high difference between the two ratios ($p \geq 0.05$) (Table 3). The inverse relation between age and the incidence of this parasite has been confirmed by many previous studies including [26] in Al-Qadisiyah Province. This is due to fact that the small animals were more receptive to infection because of incomplete immune system and lack of efficiency, and also exposure to large numbers of parasitic Oocysts Produced with newborn animal feces.

Table 2 :Distribution of infected samples and percentage of infection with *C. parvum* according to sex of animal

The animals	Number of samples Infected	Males		Females	
		Number of samples	%	Number of samples	%
Sheep	53	28	52.9	25	47.1
Cows	60	29	48.4	31	51.6
Goat	46	20	43.5	26	56.5
Chicken	36	17	47.3	19	52.7
Total	195	94	48.2	101	**51.7
Significant variations					Less than 0.001

** = high significant

Table 3: Number of infected samples and percentage of infection with *C. parvum* by according of animal's ages

Age	Small ages (1 month -2 years)			Large ages (3-5) years		
	Number of examined Samples	Number of infected Samples	%	Number of examined Samples	Number of infected Samples	%
Sheep	83	42	50.6	68	17	25.0
Cows	70	39	55.7	74	20	27.0
Goat	63	30	47.6	61	13	21.3
Chicken	78	26	33.3	67	8	11.9
Total	492	137	**46.5	270	58	21.4
Significant variations						Less than 0.001

** = high significant

There were significant differences between the rates of infection in rural areas 44.9% and urban areas 21.1% and all types of animals examined (Table 4) and this is consistent with what Khalil *et al.*, [22] found. This is due to the high prevalence of the disease in areas where the sources of infection come from grazing animals such as sheep, goats, cattle, water contaminated with parasite Oocysts and contaminated food, spread and movement of rodents within breeding fields and the spread of insects that help transport the infection compared to urban areas where the sources of transmission and spread are limited.

Table 4: Number of infected samples and percentage with *C. parvum* by area of animal presence

Area	Rural			Urban		
	Number of examined Samples	Number of infected Samples	%	Number of examined Samples	Number of infected Samples	%
Sheep	82	41	50.0	66	15	22.7
Cows	86	40	46.5	58	15	25.8
Goat	72	33	45.8	60	10	16.6
Chicken	78	29	37.1	62	12	19.3
Total	318	143	**44.9	246	52	21.1
Significant variations						Less than 0.001

** = high significant

The results showed that the water extract of *Zygophyllum fapago* plant had a significant effect on parasite growth in the cultural media. The concentration of 5 mg / ml killed the parasites by 100% after one day of treatment. Moreover, the concentrations (0.5, 1.5, 2.5, 3.5) mg /ml led to an inhibition effect of (48.7, 61.4, 75.5, 79) % respectively, and after 2 days, the parasite killing rate increased to (56.2, 70.4, 80.1, 85) % respectively, while after 4 days of the treatment, the killing rate by the above concentrations reached (90.2, 97.1, 100) % respectively. The high parasite killing rate was observed significantly after 5 days of treatment, the concentration of 0.5 mg / ml was inhibition killing 95.5%, while the rest of the concentrations had a complete inhibitory effect on the growth of parasites with a killing ratio 100% (Table 5). This corresponds to the study by Eibada [27] which used the extract of garlic plant and pomegranate peel to study their effect on the growth of the parasite.

Table 5 Percentage of parasite killing *C. parvum* in the culture media using different concentrations of water extract of *Z. fapag* plant

Period (Day) / Concentrations mg / ml	Percentages of parasite killing %				
	1	2	3	4	5
Control	0.0	0.0	0.0	0.0	0.0
0.5	48.7	56.2	73.3	81.0	95.5
1.5	61.4	70.4	80.6	90.2	100
2.5	75.5	80.1	89.9	97.1	100
3.5	79.0	85.0	92.4	100	100
5	*100	100	100	100	100
Mean	72.9	78.3	87.3	93.6	99.1

* Concentration 5 mg / ml is most effective in parasite killing after one day of treatment with water extract of plant

The inhibitory effect of plant extract on parasite growth is due to its containment on many alkaloids, glycosides and tripartite compounds and volatile oils that affect the

internal enzymes of the parasite and lead to the disruption of the centers of enzyme making and cell death. Moreover, prevents the passage of ions through the cell membrane and influence the biological activities of the parasite by disabling the enzyme acetylcholine, which controls all the physiological functions of the parasite and this leads to the death of the parasite [28].Table (6) shows the chemical reagents used on *Z. fapago* to determine the effective compounds.

The results showed that the plant contains many effective compounds, namely alkaloids glycosides, saponins, and phenols, which affected the growth of the parasite and led to its killing 100%, Which have a key role in inhibiting the growth and elimination of parasites. This is what Mustafa [29] has pointed out namely, that many plants contain substances and compounds that are effective and have an effective effect on bacteria, fungi and parasites.

Table 6: Chemical reagents on bioactive compounds in extract of *Z. fapago* plant

Active compounds	Type of Reagents	Reagents Guide	Result Reagents
Glycosides	Reagent Benedict	Red color	+
Flavonoids	Potassium hydroxide 50%	Yellow precipitate	-
Phenols	Ferric chloride 1%	Greenish green sediment	+
Alkaloids	Meyer Reagent	White deposit	+
Terpenes	Sulfuric acid concentrates with chloroform	Brown deposit	-
The Saponins	Mercuric chloride 1%	White precipitate	+
Tannins	Lead acetate 1%	White gelatin deposits	-
Resins	Acid 4% HCL	The turbidity	-

(+) the presence of an effective compound (-)Lack of effective compound

Conclusions

- The infection of *Cryptosporidium parvum* parasite was recorded for the first time in animals of Al-Anbar province.
- There is no significant difference in the incidence of parasitic infection between
- Males and females.
- High incidence of parasitic infection in small age groups.
- The infection rate in rural areas was higher than in the urban areas.
- The study showed the effect of the water extract of *Zygophyllum fapago* plant on the growth of the *C. parvum* parasite in the cultural media, the concentration 5 mg / ml recorded the greatest effect with a killing rate 100% after one day of treatment.

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