

## Hepatic Coccidiosis In Rabbits And Comparative Study On Treatment With Herbal Drug

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### ABSTRACT

Twenty five Rabbits were collected and when examined microscopically showed, *Eimeria stiedae* Oocyst from both fecal and liver smears (40%). Naturally infected rabbits showed emaciation, diarrhea and liver nodules yellowish white in colour. Forty rabbits two months of age were divided into 4 groups one kept as control, the last 3 groups were infected with the isolated oocyst and each rabbit received 100000 sporulated oocyst per 3ml. orally infected group only showed severe signs as diarrhea, decrease in body weight, loss of appetite and 30% mortality. Liver damage and enlargement, liver showed hepatomegally, yellowish white nodules on the surface and severe histopathological lesions in liver and bile duct. While the last 2 groups infected and treated 30 days post infection. Group treated with Neem extract 4% (Herbal anticoccidial). Showed normal body weight which increased post treatment, and improvement in liver function (AST and ALT) while group treated with Toltrazuril showed significant increase in body weight more than the control group. Also liver function improvement was more responded with no symptoms in both treated groups.

### INTRODUCTION

Hepatic coccidiosis (*Eimeria stiedae*) is one of the most pathogenic coccidian protozoans in domestic Rabbits causing severe coccidiosis and increased mortality (1-4).

Rabbit industry progress rapidly in Egypt to overcome the excessive demand for animal protein. The Rabbits production must see the light from the opened doors, moreover Rabbits are used as laboratory animals and for fur production. The Rabbits meats are highly digestible because it is rich in essential amino acids particularly lysine, valine, leucine, methionine and threonine (5).

Hepatic coccidiosis is a primary disease of young Rabbits and causes severe infection, outbreaks and deaths. It's causative agent is *E. stiedae* and it's developmental forms are found in the bile duct epithelial cells. While most infections are mild and these infections are

often clinically inapparent, severe infections can result in progressive emaciation, liver enlargement, diarrhea, ascites and leading finally the death (6).

Rabbits, besides their use as laboratory animals. Are raised for a variety of commercial purposes meat and fur. They are efficient converters of vegetable protein into high quality animal protein (7).

*E. stiedae* infection of rabbits caused a considerable number of changes in the metabolism of lipids, proteins and glucose, which is likely due to direct effects of liver cirrhosis on normal body function (8).

Treatment of infected Rabbits with 2.5% Toltrazuril (Baycox) at 25 ppm in water for 2 days was highly effective. The Oocysts is appeared in the feces on day 6 after treatment. No mortality was seen in treated Rabbits while

all the infected untreated Rabbits died within a period of 7 days (9).

Medicinal plants were part and parcel of human society to combat diseases, from the dawn of civilization. Generally, complementary medicine for treating or preventing influenza or influenza-like illness in human and reducing coccidial Oocyst burden in broilers (10,11).

## MATERIAL AND METHODS

### Naturally infected Rabbits

Twenty five emaciated Rabbits were collected from the markets, fecal samples and liver specimens were positive for *E. Stiedae* oocysts in 40% of collected rabbits.

### Sporulation and counting of the oocyst

Isolated *E. stiedae* Oocysts were collected by using the flotation technique ; (12), using zinc sulphate (33%). The Oocysts were mixed with 2.5% potassium dichromate

solution and placed in a thin layer 2mm depth in Petri- dishes and kept in incubator at 26C°.

Examination daily up to five days to detect Oocyst sporulation. Counting of the sporulated oocyst was carried out with the McMaster slide technique and dose infection was adjusted to 100000 sporulated Oocyst per 3ml.

### Experimental design: table (1)

Forty healthy non infected rabbits were used in experimental infection, were divided into four equal groups (10 Rabbits per group) aged 2 month, the first group kept as control (non infected non treated), groups 2, 3, 4 were infected with *E. stiedae* sporulated oocyst 100000/3ml for each rabbit orally. Rabbits in group 3 were treated with Aqueous Neem extract 4% in drinking water for 5 days, 21 days post infection. While Rabbits in group 4 were treated with Toltrazuril 2.5% in drinking water for 2 days at 21 days post infection, then all rabbits in all groups were sacrificed at 30 days post infection for biochemical examination histopathological examinations.

**Table 1. Experimental design**

Group	No. of rabbits	Dose/3ml	Infection	Treatment	
				Neem	Toltrazuril
(1) Control	10	-	-	-	-
(2) Infected, non treated	10	100000 sporulated Oocysts of <i>Eimeria stiedae</i>	+	-	-
(3) Infected and treated with aqueous Neem leaves extract 4%	10	100000 sporulated Oocysts of <i>Eimeria stiedae</i>	+	+	-
(4) Infected and treated with Toltrazuril 2.5%	10	100000 sporulated Oocysts of <i>Eimeria stiedae</i>	+	-	+

The experimental rabbits were examined for liver lesions and blood was collected and sera were separated for liver function test (AST & ALT) also body weight was estimated.

Pieces of hepatic tissue, heavily infected with coccidiosis (apparent normal rabbits) was fixed in 10% formalin and paraffin sections of 5 microns (TS) thickness were prepared, stained with Haematoxylin and Eosin (13) and examined microscopically.

# RESULTS

Twenty five naturally infected rabbits were examined which showed emaciation, diarrhea and off food. Microscopically fecal smears showed *E. stiedae* Oocyst. Liver examination revealed hepatomegally with whitish yellow nodules and microscopical examination showed *E. stiedae* Oocyst and the percentage of positive cases was 40% (Fig. 1).

## Sporulation of Oocyst

Sporulation was completed in 2.5% potassium dichromate within five days and the sporulated Oocyst was oval in shape with cap and double wall, each contains four sporocysts.

## Experimental infection

Forty, 2 month old healthy rabbits were divided into four groups, G1 (control) while G2, G3, G4 were infected which showed severe symptoms, retarded growth, diarrhea off food, ruffled fur and 30% mortality, livers were enlarged with yellowish white nodules

and distended gall bladder and microscopical examination showed *E. stiedae* (Figs. 2 and table 2).

Treatment , 21 days post infection in G3 with aqueous Neem extract 4% showed significant increase in body weight (Table 3) and lower mortality. While treatment of group four (G4) with toltrazuril 2.5% showed complete releave with no symptoms or mortalities and highly significant increase in body weight (Table 4).

Liver function (AST and ALT) showed increase in both enzymes in rabbits of G2, while in both groups G3 and G4 showed decrease in AST and ALT post treatment and nearly similar to control (Tables 3 and 4).

## Microscopical examination

Severe congestion of central veins were observed, severe hyperplasia of the lining epithelium of the portal areas were clear forming finger – like projections in the bile duct lumen, containing fairly numerous developmental stages of *E. stiedae* (Figs. 3, 4, 5, 6).

Table 2. Results of infected non treated Rabbits (G<sub>2</sub>)

Animal No.	Body weight			Liver function of infected & non treated					
	A Before infection	B After infection	Percent % (1)	AST			ALT		
				Before inf.	After inf.	Percent % (2)	Before inf.	After inf.	Percent % (2)
1	1500	1085	27.66	37	140	278.37	47	91	93.61
2	1490	1075	27.85	35	140	300.00	45	92	104.44
3	1480	1075	27.36	36	130	261.11	45	98	117.77
4	1495	1085	27.42	37	140	278.37	45	90	100.00
5	1495	1080	27.75	35	140	300.00	40	90	125.00
6	1500	1085	27.66	35	135	285.71	40	95	137.5
7	1490	1070	28.18	35	140	300.00	40	92	130.00
8	1485	-	-	-	-	-	-	-	-
9	1490	-	-	-	-	-	-	-	-
10	1495	-	-	-	-	-	-	-	-

A= Body weight before infection.

B = Body weight after infection.

(1) = Percentage of decrease in body weight.

(2) = Percentage of increase in AST and ALT.

(-) = 3 Rabbits were died after infection.

Table 3. Result of infected and treated Rabbits with Neem extract (G<sub>3</sub>)

Animal No.	Body weight		Liver function of infected & treated						
	A Before treatment	B After treatment	Percent % (1)	AST		Percent % (2)	ALT		Percent % (2)
				Before treatment	After treatment		Before Treat.	After Treat.	
1	1080	1500	38.88	140	49	65%	91	121	32.96
2	1085	1510	39.17	140	50	64.28	92	120	30.43
3	1090	1500	37.61	130	48	58.57	98	118	20.40
4	1080	1520	40.74	140	49	65.00	90	120	33.33
5	1085	1520	40.09	140	52	62.85	90	120	33.33
6	1090	1500	37.61	135	57	57.77	95	120	26.31
7	1090	1530	40.36	140	55	60.71	92	119	29.34
8	1085	1520	40.09	130	53	59.23	98	122	24.48
9	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-

A= Body weight before treatment.

B = Body weight after treatment.

(1) = Percentage of increase in body weight.

(2) = Percentage of increase or decrease in liver enzyme (AST,ALT).

(-) = 2 died rabbits after infection.

Table 4. Results of infected and treated Rabbits with Toltrazuril (G<sub>4</sub>)

Animal No.	Body weight		Liver function of infected and treated						
	A before treatment	B After treatment	Percent % (1)	AST		Percent % (2)	ALT		Percent % (2)
				Before treatment	After treatment		Before Treat.	After Treat.	
1	1085	1570	44.70	140	52	62.85	121	59	51.23
2	1080	1580	46.29	140	50	64.28	120	57	52.5
3	1080	1565	44.90	130	52	60.00	118	57	51.69
4	1080	1570	45.37	140	52	62.85	120	59	50.83
5	1080	1560	44.44	140	50	64.28	120	60	50.00
6	1085	1550	42.85	135	55	59.25	120	65	45.83
7	1090	1560	43.11	140	50	64.28	119	60	49.57
8	1085	1560	43.77	130	53	59.23	122	59	51.63
9	1095	1570	43.37	130	52	60.00	120	59	50.83
10	1080	1570	31.21	135	52	61.48	120	57	52.5

A= Body weight before treatment.

B = Body weight after treatment.

(1) = percent of increase in body weight.

(2) = Percent of decrease of liver enzymes (AST) (ALT).

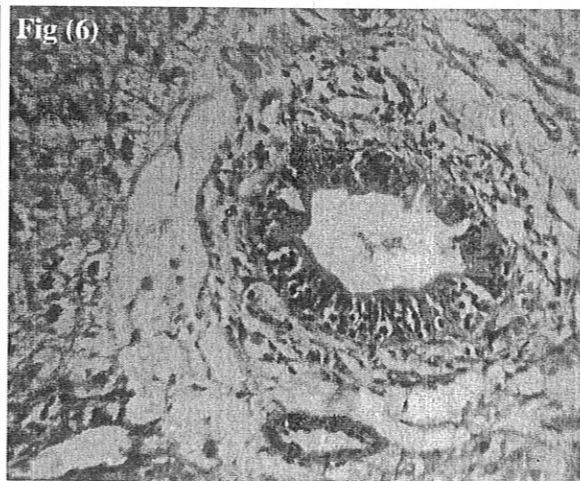
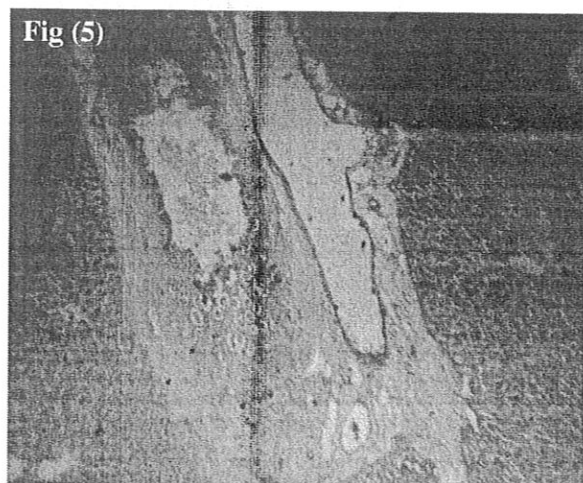
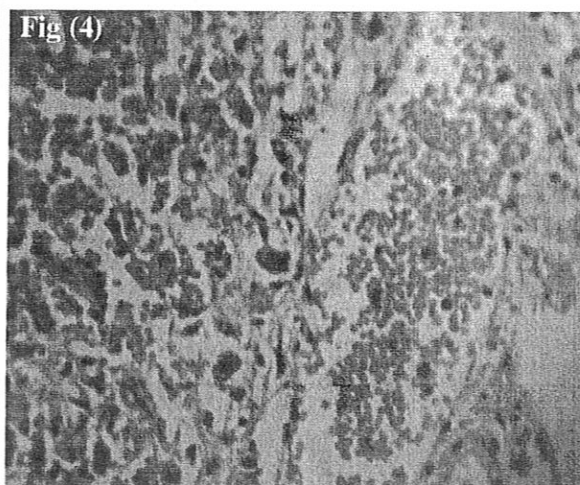
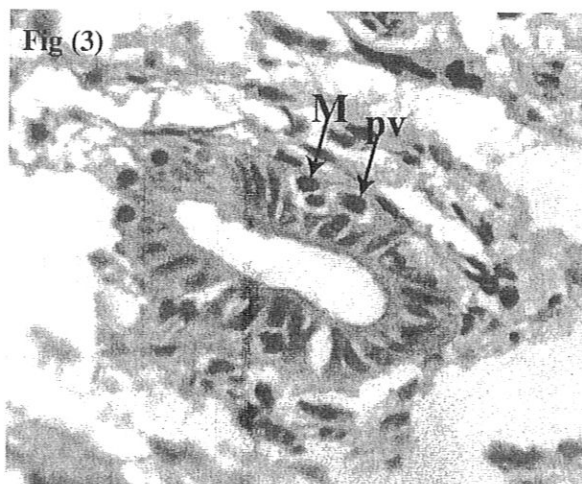
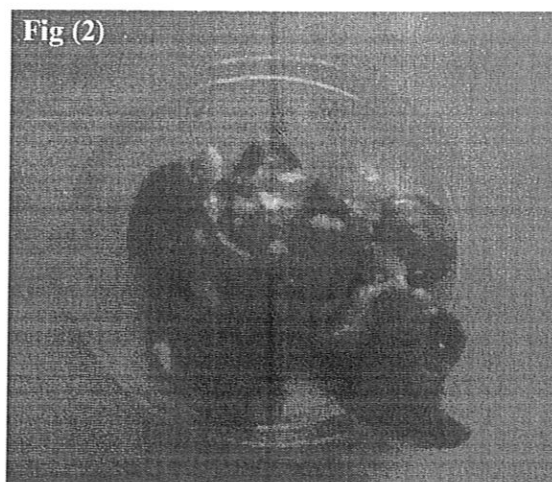
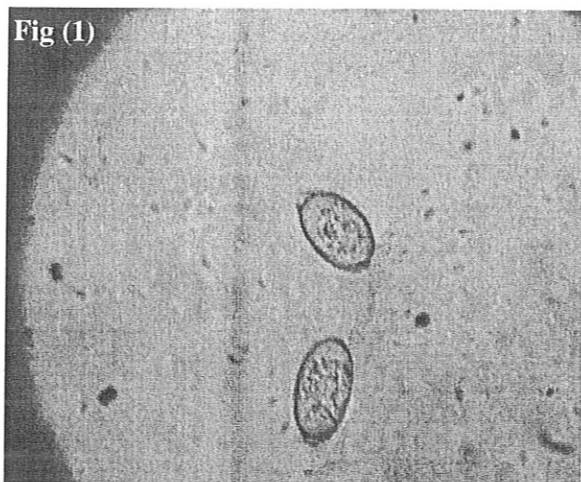


Fig.1. Photomicrograph of fresh non –sporulated oocyst of *Eimeria stiedae* oocyst (x1500)

Fig.2. Liver infected by *Eimeria stiedae*

Fig.3. Photomicrograph of 1st generation uninucleated meront (M) within a large parasitophorous vacuole (pv) (H & E x 1200)

Fig. 4. Showing congestion of the hepatic blood vessels together with haemorrhage (H&E x 520)

Fig. 5. Showing massive periductal necrosis with developmental stages of *Eimeria* (H&E x 130)

Fig. 6. Showing periductal fibrosis and hyperplasia of lining epithelium of bile duct (H&E x 520)



## DISCUSSION

Hepatic coccidiosis is a primary disease of young rabbits and causes severe infection, outbreaks and deaths (6) it's developmental stages are found in the bile duct epithelial cells *E. stiedae* causing hepatic coccidiosis is a potential threat to the rabbit industry 14.

In our study, the observed clinical signs on twenty five collected naturally infected rabbits showed decreased growth rate, anorexia, emaciation, diarrhea, icterus and rough hair coat, the results agree with (6,7) . who stated that severe infection can result in progressive emaciation, liver enlargement, diarrhea, and leading finally to death.

The post-mortum examination of the collected naturally infected rabbits revealed the following gross lesions, mild ascitis, multiple 1-3 mm diameter, discrete to coalising and may reach to 1cm in diameter, yellow to white nodules in the liver in about 40% of examined rabbits.

The obtained results agree with (16,17) who mentioned that the gross lesions were hepatomegally with yellow white nodules, ascitis and distended gall bladder (18). found that 32.24% of examined rabbits were infected with *E. stiedae*. Concerning the laboratory diagnosis of hepatic coccidiosis by using light microscopic examination showed that *E. stiedae* in rabbits are oval in shape, yellowish orange in colour, showing a micropyle. These results agree with (12,19-22), who mentioned that *E. stiedae* oocyst were ovoid to oval, their walls were smooth, yellowish to orange in colour, their anterior end was narrower than the posterior one and contain a micropyle.

After sporulation in 2.5% pot. Dichromate, the experimentally infected rabbits showed emaciation, diarrhea, jaundice and paralysis before death these results agree with (23-25), Who mentioned that the clinical signs in experimentally infected rabbits were loss of appetite, staggering gait and paralysis of legs, diarrhea, jaundice and convulsions followed by death.

Regarding to the macroscopical picture of liver post infection showed enlargement of the liver with yellowish white nodules on the surface and throughout the parenchyma, *E. stiedae* Oocyst was detected microscopically from liver nodules these results agree with (15, 17, 26-28) , who mentioned that the liver lesions were hepatomegally with liver damage and yellowish white nodular abscess like lesions.

Mortality percentage was high (30%) in experimentally infected rabbits orally with 100.000 sporulated oocysts for each rabbit per 3ml. These results agree with (29) observed 42% mortality in 2 month age rabbits, (26) in France where the percentage of mortality was 22.5% while in Brazil was 48%- 64%, also (30), mentioned that young rabbits experimentally infected with 10.000 or 100.000 Oocysts had 40% and 80% mortality, respectively.

Rabbits in all groups ( $G_1$ ,  $G_2$ ,  $G_3$ ,  $G_4$ ) were weighed before infection and after infection and after treatment which showed significant decrease in body weight 21 day post infection  $G_2$  which ranged from (1070-1085). These results agree with (31), who observed that reduction of weight gain from the 8<sup>th</sup> day of infection and actual loss from the 15<sup>th</sup> day in infected animals. While  $G_3$  showed increase in body weight which infected and treated with Neem extract and ranged from (1500-1530), these results agree with (32).

Significant increase also in body weight was observed in  $G_4$  that was treated after infection with toltrazuril, which ranged from (1550-1580) when compared with the infected group ( $G_4$ ) before treatment which ranged from (1080-1090) these results agree with (9,33).

Liver function test was carried out to detect the effect of *E. stiedae* infection on liver enzymes AST and ALT, the results in  $G_2$  showed very highly significant increase in AST values which ranged from 130-140 when compared with the control group 35-37, while the ALT values also showed highly significant values 90-98 and the control values ranged

from 40-47. These results agree with (34-36) who stated that a highly significant increase in AST and ALT was observed and the increase in ALT may indicate liver inflammation and necrosis.

While liver function after treatment with Neem extract at 21 days post infection, the values of AST and ALT showed significant decrease in AST and highly significant increase in ALT values (48-57), (118-122) respectively which indicate liver damage milder than the infected group, the effect of Neem extract was clear as it reduced coccidial Oocysts and reduced mortality from 30% in control into 20% with Neem extract and improve body weight (Table 4), these results agree with (11).

Treatment with toltrazuril orally at 21 day post infection revealed significant decrease in both AST and ALT values, (50-55) and (57-60) respectively when compared with the infected group (130 – 140), (90 – 98) which indicate milder liver damage than neem extract, also toltrazuril treatment reduced oocyst and improve body weight (Table 5). These results agree with (9,33).

Histopathological examination of infected liver revealed the following pictures in G<sub>2</sub> infected only figures from 6-21 showed various degrees of liver damage and congestion of hepatic blood vessels with perivascular fibrosis and the congestion in some cases was associated with extravasated blood into the hepatic tissue. In some cases focal coagulative necrosis of the hepatic tissue, mild cloudy swelling. Also *E. stiedae* Oocyst within the epithelial cells lining the bile duct detectable in most cases, associated with massive necrosis and mononuclear cells infiltration. Marked hyperplasia of epithelial lining of bile duct was detected. These results agree with the histopathological finding of (15, 18, 23, 35,37,38).

While histopathological changes in G<sub>3</sub> which infected and treated with Neem extract, the hepatic lesions showed mild lesions than that recorded in the infected group only (G<sub>2</sub>),

as only congested liver with petichelial haemorrhages were detected.

It is clear that Neem extract relief tissues from coccidial effect and used in the future as potent herbal anticoccidial in hepatococcidiosis (11). But the mild liver changes may be due to the low doses of Neem extract in water and this point needs more studies to adjust the suitable doses.

While the use of Toltrazuril in treatment of infected rabbits showed no characteristic histopathological lesions and the liver was normal as that detected in the control group.

**In conclusion**, it is clear that the incidence of hepatic coccidiosis in rabbits was high (40%) and showed economical losses in rabbits due to severe liver damage, high mortality (30%) and emaciation. Treatments depends on good hygiene and the use of drugs as toltrazuril as well as recent herbal anticoccidial agues Neem extract which is a new anticoccidial and used for the first time in Egypt, but it needs more studies to adjust the suitable dose and course of treatment.

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### الملخص العربي

الكوكسيديا الكبدية في الأرانب ودراسة مقارنة علي العلاج بعلاج عشبي

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\*\*\* المركز القومي للبحوث.

تم تجميع خمسة وعشرين أرنباً وبالفحص الميكروسكوبي تبين وجود حويصلات الكوكسيديا الكبدية (*E. stiedae*)، من مسحات كل من البراز والكبد بنسبة (٤٠ ٪). أظهرت الأرانب المصابة بشكل طبيعي وجود هزال وإسهال مع وجود آفات مرضية بالكبد بيضاء مصفرة اللون.

تم استخدام أربعين أرنب خالية من الكوكسيديا وذلك عن طريق الفحص الميكروسكوبي عند عمر شهرين، تم تقسيمها إلى ٤ مجموعات متساوية كل مجموعة تحتوي علي عشرة أرانب، الأولى ضابطة غير معدية وغير معالجة والمجموعة الثانية معدية فقط باستخدام حويصلات معزولة تم وضعها في محلول بوتاسيوم دايكرومات ٢,٥ ٪ للحصول علي الطور المعدي وتم تحديد الجرعة لتكون ١٠٠٠٠٠ لكل ٣ سم من المحلول تعطي لكل أرنب عن طريق الفم، أما المجموعة الثالثة فقد تم عمل العدوي كما في المجموعة الثانية بالإضافة إلي العلاج بمركب عشبي منقوع أوراق النيم ٤ ٪ بعد العدوي بشهر أما المجموعة الرابعة تم عمل نفس العدوي ثم العلاج باستخدام عقار تولترازوريل ٢,٥ ٪.

وأظهرت النتائج التالي: بالنسبة للمجموعة الثانية ظهرت عليها أعراض الضعف والهزال والإسهال بالإضافة إلي تلف الكبد وتضخمه مع ظهور آفات مرضية بالكبد بيضاء مصفرة اللون علي سطح الكبد، وبالدراسة الهستوباثولوجية وجد تأثير خطير للكوكسيديا علي الكبد والقنوات المرارية، وكانت نسبة الوفيات ٣٠ ٪، كما أظهرت النتائج للموجعتين الثالثة والرابعة وزن الجسم الطبيعي والتي زادت بعد العلاج، وتحسن في وظائف الكبد (AST و ALT)، في حين أظهرت المجموعة التي تلقت العلاج بـ Toltrazuril زيادة معنوية في وزن الجسم أكثر من المجموعة الضابطة بالإضافة إلي تحسين وظيفة الكبد مع عدم وجود أعراض للإصابة بالكوكسيديا.