

## **EXPERIMENTAL INFECTION OF CALVES BY *GIARDIA* *LAMBLIA* CYST ISOLATION FROM HUMAN**

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### **ABSTRACT**

Conducted an experimental study on the possibility of a calf infection by *Giardia* parasite isolated from human. Six calves were selected from the field of the Faculty of Veterinary Medicine at the University of Basrah and were divided into two groups, the first was a control group consisting of 2 calves (n=2), and the second was group that infected and composed of four calves (n=4). After the experiment done the percentage of infection was 100%. This study determined the amount of the dose that can cause infection to the calves and are (7-21) cysts which were approach to those causing infection to human. The study also identified the incubation period for the giardiasis disease in calves, which were (7-10) days. This study is the first one in the Basrah and Iraq.

### **INTRODUCTION**

*Giardia lamblia* is a binucleate flagellated protozoan parasite that infects the upper intestinal tract of human and many animals' species (1). It was one of the first protozoan's which discovered by Leeuwenhoek in 1681 in his own diarrheal stool (2). *Giardia* is an intestinal flagellate that infects a wide range of vertebrate hosts. The genus currently comprises six species, namely: *G. agilis*, *G. ardeae*, *G. duodenalis*, *G. microti*, *G. muris*, and *G. psittaci*, which are distinguished on the basis of the morphology and ultrastructure of their trophozoites (3). In Asia, Africa, and Latin America, about 200 million people have symptomatic giardiasis with some 500,000 new cases reported each year (4). It was also a frequently encountered parasite of domestic animals, especially livestock, dogs and cats, and numerous species of wild mammals and birds, and even fish have been documented as hosts of *Giardia* (5). Nowadays *Giardia* is recognized as the most common parasitological cause of

diarrhea in human patients, with an estimated 280 million infections per year, and is a major concern to drinking water authorities, as it is a frequently diagnosed waterborne infection (6). Similarly, giardiasis is the most common parasitic infection in companion animals (6). Because of the impact on socio-economic development, especially in developing countries, *Giardia* is included in the “Neglected Disease Initiative” of the World Health Organization (7; 8). In laboratory animals, such as mice and gerbils, the pathology caused by infection has been described and is widely accepted (9), where as in companion animals and food animals the pathological changes have not been well documented (10). In both humans and animals, the clinical outcome of a *Giardia* infection in animals is highly variable and infection can result in either acute or chronic diarrhea, nausea, weight loss, and hypersensitivity but asymptomatic infections are also known to occur frequently (3). It has been reported worldwide in farm animals, although prevalence data are mainly available for cattle, and to a lesser extent for other ruminants (3). In food-producing animals and in pets, the infection can reduce weight gain, and may become a concern for zoonotic transmission (8; 11). The symptoms are characterized by acute watery diarrhea, dehydration, weight loss and abdominal discomfort (3). *Giardia* is the major human intestinal pathogen globally, and the common cause of diarrhea in developed countries (13). The infection spreads via the fecal oral route. It is generally caused by contaminated drinking water and only 10 cysts is enough to cause infection (5 ; 14). The life cycle of *G. intestinalis* can be divided into infective cysts and vegetative growing trophozoites (7). The cyst is highly resistant to the surrounding environment, dormant life form and important for disease transmission whereas, trophozoite represents the motile, vegetative form, colonizing the small intestine and cause diarrhea and malabsorption (7). In order to complete the life cycle, the trophozoites form cysts in the small intestine and are passed through feces (7). Cysts can survive outside the host for several months (7). This intestinal protozoan has been found in a wide range of mammals and has been accepted as a zoonotic agent (12; 15).

## **MATERIALS AND METHODS**

### **Samples collection:**

Samples of stool taken from patients complain of diarrhea, abdominal discomfort, nausea and abdominal cramp.

All samples collected on sterilized cups and taken up to laboratory and diagnosis by:

1- Direct smear: (16)

a) Lugol's iodine.

b) Normal saline: to detection for trophozoite.

2- Concentration methods: formol-ether sedimentation (16).

The positive stool samples divided into three groups' according to parasite density (19):

- i. Heavy infection: 6 – 8 cysts or trophozoites per microscopic field.
- ii. Moderate infection: 3 – 5 cysts or trophozoites per microscopic field.
- iii. Mild infection: 1 – 2 cysts or trophozoites per microscopic field.

Positive stool samples of human are collect and cyst purification done.

### **Cysts purification:**

The cyst purification method of the present study was done by slightly modifying the procedure of (17).

Stool samples were diluted (1:10) with distilled water filtered through four layers of gauze, then through 125 µm, 90 µm aperture metallic sieves. 4 to 5 ml of the filtrate samples were placed over 3 ml of chilled 0.85 M sucrose solution in a 10 ml conical centrifuge tube. After centrifugation at 1800 r.p.m. for 5 minutes at room temperature, the water-sucrose interface was removed, diluted (1:10) with distilled water and re centrifuged for 5 minutes. The pellet was re suspended in 4 ml of water and the sucrose gradient was repeated. The above procedure was repeated three times. The purified cyst suspensions were used freshly in the experimental infection of calves or stored at 4 C° in distilled water until it was needed (17).

### **Cyst counting:**

The densities of cysts were quantitated by a hemacytometer method (18). Counting of cyst done as method of counting of white blood cells to determined the dose of infection, in this operation we use iodine solution as dilution in spite of solution Turkey.

The resulted of cyst count determined by hemacytometer method, a cover slip was placed over the counting chamber and the platforms were flooded with 10 µl / platform. The 4 corner squares (1mm<sup>2</sup> each) on each platform were counted and cyst density was calculated as follow (18):

$$\frac{\text{cyst no.}}{4 \text{ mm}^2} \times \frac{10}{1 \text{ mm}} \times \frac{1 \text{ mm}^3}{\text{ml}} = \text{cysts/ml}$$

Cyst no. = number of cyst in 4 chambers

4 mm<sup>2</sup> = total area

1 mm<sup>3</sup> = total volume (18).

$$\frac{3 \text{ cyst}}{4 \text{ mm}^2} \times \frac{10}{1 \text{ mm}} \times \frac{1 \text{ mm}^3}{\text{ml}} = 7 \text{ cysts/ml}$$

3 = number of cyst in 4 chamber were count

3 cc of counting dose use for inoculation calves

∴ 7 X 3 = 21 cyst in 3 cc.

### **Experimental calves:**

Six calves aged 13 – 18 months were chosen from the field of the Faculty of Veterinary Medicine at the University of Basrah. Make two groups first control (n=2) and second was infected group (n=4). Calves are isolated and examine generally, clinically and laboratory by light microscope in power 10 X and 40 X by above methods since one month before experimenting, then examine two weeks later , one week , one day respectively to be sure that calves were free from any infections.

### **Inoculation calves:**

3 cc of counting dose are diluted in 250 ml of sterilized distal water, then via gastric intubation to 4 calves (infected group), each one of 4 calves of infected group were inoculate 3 cc of counting dose contain 21 cysts. Administrated cows are

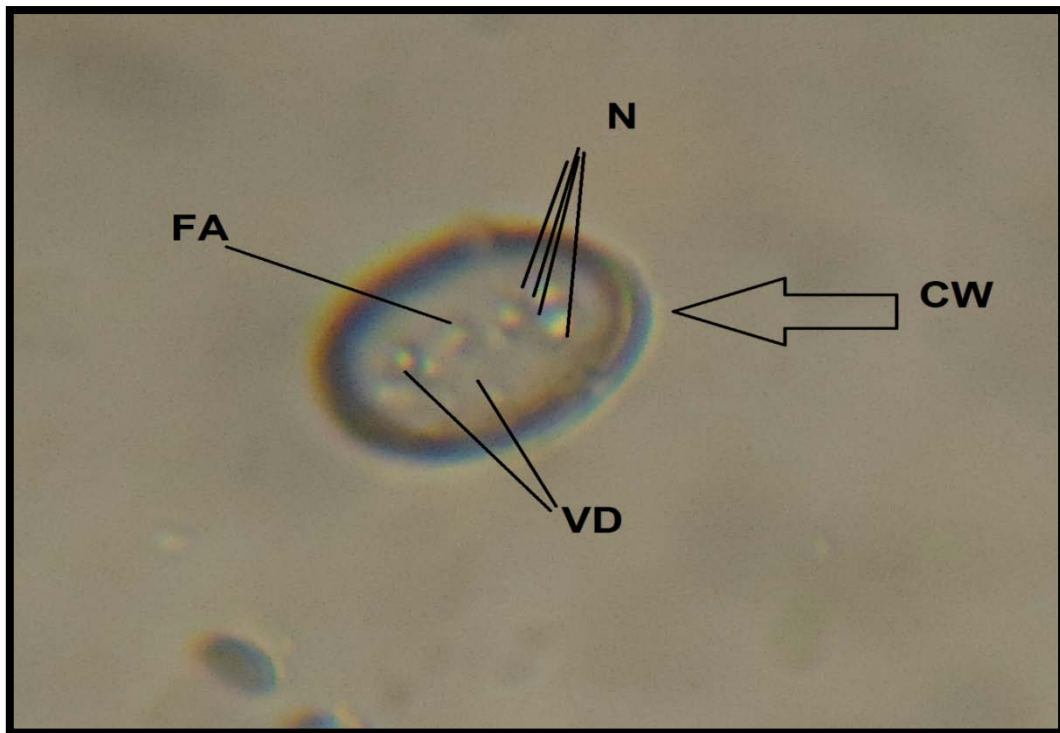
examined two days after inoculation daily. These isolated from control group which not inoculated with cyst.

## RESULTS

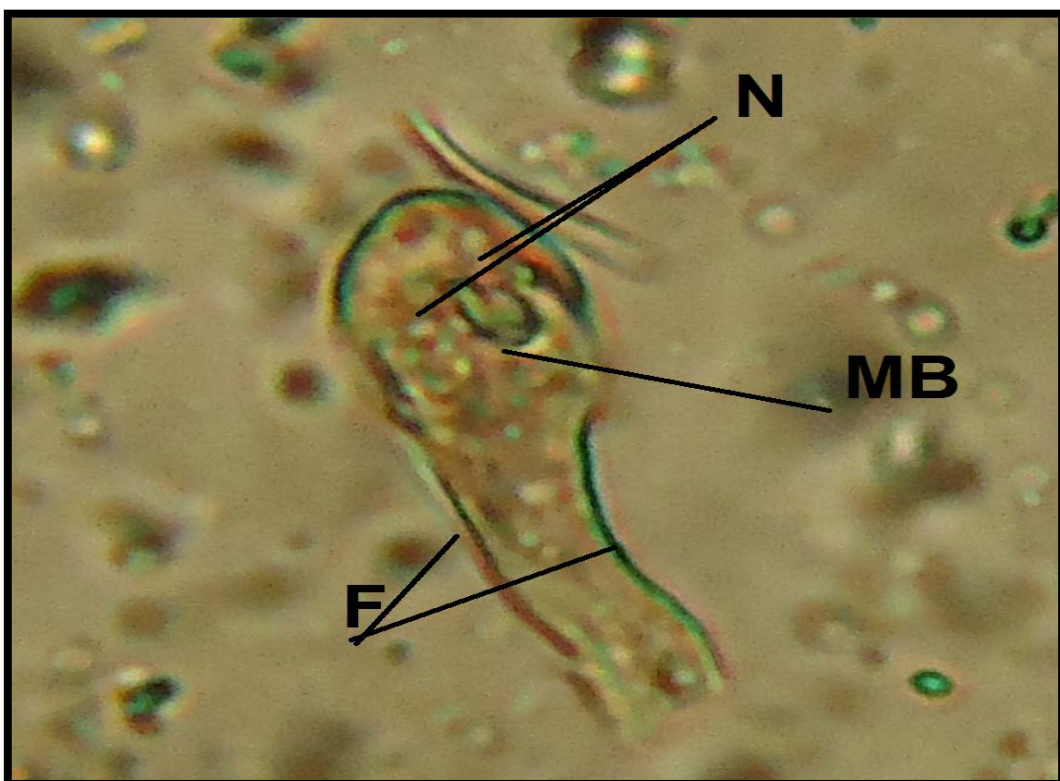
In this work all members of infected group (n=4) show infection with giardiasis, so the infection rate is 100%. The infected dose was identified which is 7 – 21 cysts in 3 cc were inoculated and it calculated by hemacytometer method in cyst counting. Daily examination of inoculation calves feces show that after 7 day of inoculation cyst (Fig 1), trophozoites (Fig 2) was appear without symptoms. Incubation period is from 7 – 10 days in calves (table 1).

**Table 1: *Giardia* infection appearance after inoculation in experimental calves.**

Calf no		Days of infection appear after inoculation									
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>	10 <sup>th</sup>
Infected group	1			-	-	-	-	+	+	+	+
	2			-	-	-	-	-	-	-	+
	3			-	-	-	-	-	+	+	+
	4			-	-	-	-	+	+	+	+
Control group	1			-	-	-	-	-	-	-	-
	2			-	-	-	-	-	-	-	-



**Figure 1: Cyst of *Giardia* in direct smear ( 16 X 40 ) 3D ( nuclei: N, Flagellar axonemes: FA (axostyle) ,Ventral disc :VD , cyst wall : CW ).**



**Figure 2: *Giardia* Trophozoite (16 X 40 ) 3D ( Binucleate : N, Four pairs of flagella: F, Two “claw-hammer” shaped median bodies: MB).**

## DISCUSSION

The results of this study refer that transmitting of *Giardia lamblia* can occur between human and calves, this give an agreement with *Giardia* can be transferred from domestic animals to human beings and is a potential zoonotic agent (1), *G. duodenalis* isolated from humans and animals is genetically comparable which confirm its zoonotic nature (20).

In this study determined the lower dose that can cause infection which is 7 – 21 cysts and this result was nearly closed to the dose can cause human giardiasis, and this agree with (3), the cyst persists for months in cold fresh water, and ingestion of as few as 10 cysts can initiate infection, the *Giardia* infection can be caused by ingestion of 10-100 cysts or less numbers in human (22).

This study show that incubation period of giardiasis in calves is 7 – 10 days and this nearly to that result of (22) is incubation period in human 8 – 10 days , The incubation period 8-10 days was observed in cattles and humans (21).

### الإصابة التجريبية للعجول بطفيلي الجيارديا المعزول من البشر.

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### الخلاصة

أجريت دراسة تجريبية حول إمكانية إصابة العجول بطفيلي الجيارديا *Giardia lamblia* المعزول من البشر. اختيرت ستة عجول من الحقل التابع لكلية الطب البيطري في جامعة البصرة و قسمت إلى مجموعتين الأولى هي مجموعة السيطرة و مكونة من عجلين و الثانية هي المجموعة التي أصيبت و مكونة من أربعة عجول. وبعد إجراء التجربة كانت نسبة الإصابة 100 %. خلال هذه الدراسة حددت كمية الجرعة التي يمكنها التسبب بالإصابة للعجول و هي ( 7 – 21 ) كيس و كانت مقارنة لتلك التي تسبب إصابة في البشر. و أيضا حددت الدراسة فترة الحضانة لمرض الجيارديات في العجول و التي كانت ( 7 – 10 ) أيام . و تعتبر هذه الدراسة الأولى من نوعها في البصرة و العراق.

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