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### Investigation and Diagnosis of *Taenia saginata* Parasite in Specific Rural Areas of Babylon Province, Iraq

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#### Abstract

The current study was conducted in the period from February 2018 to January 2021. A total of 1684 stool samples were examined to detect *T. saginata* infection in five villages of Babylon Governorate (AL-kerataa, Albo-Humaer, Al-Wardea Kharej, Alosyahe and Senjar). All samples examined by direct smear method and by kato- katz method, The results revealed that 7 (0.4%) cases were positive for *T. saginata* eggs, 5 (0.66%) males and 2 (0.21%) females, aged from 1 to 60 years old and living in the five studied villages, where the study included the effect of some basic factors such as age and gender in addition to some hematology parameter of the infected cases. This

study also showed significant variation for the average egg per gram (EPGs) of 7 egg positive samples were 8700-58,488 by kato- katz method, and 8000 -25,689 by direct smear method, the year of sample collection and hematological parameters in infected and Non-infected persons with *Taenia saginata* parasite by using statistical analysis Spss Chi square test.

**Conclusion:** The prevalence of *T. saginata* in these area even with conventional control measures indicates the importance to establish wide and multifaceted health programs to sustainably improve the quality of life of the populations living in these communities.

**Keywords:** Tap Worm, *T. Saginata*, Iraq, Kato- Katz Method, Direct Smear

#### Introduction

Intestinal parasite (IP) is one of the most widespread pathogens in the world, it infects around 3.5 billion persons a year, the majority of them are children this type of infection represents an endemic disease worldwide, especially in the tropic and subtropical regions (Fentahun *et al.*, 2019) [17]. It is mainly represented by Protozoa and Helminthes3, and they are transmitted by water and contaminated food (Amer *et al.*, 2018) [5].

The zoonotic parasite *Taenia saginata* utilizes bovines as an intermediate host (causing cysticercosis) and humans as the definitive host (causing taeniosis). The public health burden of *T. saginata* is assumed to be low, but the economic burden is large, due to the resources utilized in the detection (Saratsis *et al.*, 2019) [27]. *T. saginata*, the beef tapeworm, is an important cyclo-zoonotic cestode with a worldwide distribution. The hermaphrodite adult tapeworm develops in the human intestine and produces tens of thousands of eggs that are either excreted free or within intact, motile, proglottids in the faeces (Craig and Ito, 2007) [13]. The eggs are able to survive for several months in the environment (Taylor *et al.*, 2016) [29]. Bovids, typically cattle and buffaloes, which are of particular importance in the middle east north Africa region, are the natural intermediate hosts of the parasite, and are infected by ingestion of eggs. The oncosphere migrates *via* the bloodstream to striated muscles, where the metacestode larval stage (cysticercus) develops (Flütsch *et al.*, 2008) [18]. And human taeniosis and bovine cysticercosis are seriously understudied in West and Central Africa, the high prevalence estimates of both conditions suggest an active dissemination of this parasite in the region, calling for a concerted One Health action from public health, veterinary health and food surveillance sectors (Hendrickx *et al.*, 2019) [19].

The bovine population of the MENA region is huge, with Sudan, Egypt, Algeria, Yemen, and Syria, sorted by population rank in descending order based on 2016 data, counting among the top-producing countries in the region with an approximate population reaching almost 45 million head (including buffaloes, which are of relevance for Egypt), more than 66% of which are kept in Sudan (FAO, 2018) [16]. Both traditional and modernized bovine production systems are found in the MENA region. The traditional systems mainly cater for nomadic producers (extensive production system/mainly meat oriented) or producers

who have settled in close vicinity to cities/irrigated agricultural areas and rely on crop residues. Modernized systems largely serve intensively reared dairy cattle of both local and imported breeds (Maitah and Smutka, 2013) [23].

**Methods**

**Study Area and Human Sampling Procedures:**

The current study was conducted in the period from February 2018 to January 2021. A total of 1684 stool samples were collected from patients with diarrhea and some other gastrointestinal disorders coming to hospitals in Babylon Governorate for five villages (AL-kerata, Albo-humaer, Al-wardea kharej, Alsyaha and Senjar), where samples were kept in clean and sterile plastic containers and were examined in specialized parasite laboratories in hospitals, Fecal samples examined in less than half an hour or an hour and examination procedure include two methods:

1. **Macroscopic Examination:** The stool samples examined primarily grossly, which included the observation of quantity, consistency, color and form, blood or mucus, and some live worms (Al-Hasheme *et al.*, 2020) [2].
2. **Direct smear method:** Preparing a clean slide, using a drop of the normal saline placed on one side of the slide. Then, small amounts of the stool sample were taken from different places of the sample using a wood stick mixed with the physiological solution. then the slide was covered with the slide cover. Subsequently, the microscopic slides were examined using a microscope with magnification force of (10x, 40x, 100x) (Calderaro *et al.*, 2006) [11]. This method is estimated to contain about 2 mg of feces, and EPG can be calculated by multiplying eggs per smear by 500 (Beaver *et al.*, 1984) [7].
3. **Kato-Katz (KK) method:** The sieved feces sample (approximately 41.7 mg, 20 mg, or 50 mg depending on the size of the template) is placed on a glass slide. The preparation is covered with a piece of cellophane soaked in glycerol. Subsequently, the slide is inverted and gently pressed down resulting in a thin smear. The added glycerol serves to ‘clear’ the fecal material (fat) from around the eggs. Hookworm eggs require about 30 min for this step, while for the other species, the reading of the slide under the microscope can be done after 1 to 24 h the eggs are then counted under the microscope and the count expressed in per gram of feces (WHO, 2002; Katz *et al.*, 1972) [35, 22].  
This method produces a consistent smear of 20 mg of feces, and egg counts on one smear are converted to EPG by multiplying a constant 24 (Ebrahim *et al.*,

1997; Choi *et al.*, 2005 [12]).

**Hematological Study:**

For this purpose, 5 ml of blood samples were withdrawn following the sterilization of the methyl alcohol withdrawal zone (70%) of all patients with intestinal parasites, in addition to 25 blood samples from patients without intestinal parasites with a sterile 5 ml disposable syringe, putting 2 ml of blood drawn in a special tube containing the EDTA for the hematological parameters using the Sysmex (XN-350) dives from Japan.

**Statistical analysis**

The Statistical Analysis was performed by using Spss (2012) program to know the of difference factors in study parameters. Chi-square test was used to significant compare between percentages in our study. at  $p \leq 0.05$ .

**Results**

This study included the collection of 1684 stool samples for patients suffering from abdominal pain, diarrhea and general weakness. For rural areas of Babylon governorate from residential, agricultural and cattle breeding areas (AL-kerataa, Albo -humaer, Al-wardea kharej, Alsyaha and Senjar), for the period from (January 2018 - January 2021), and the Table 1 showing the percentage of infection for mentioned area Where the highest infection rate 1% was for the Albo -humaer region and the lowest rate 0.3% for AL-kerataa region while infection rate 0% for Al-wardea kharej region.

**Table 1:** The percentage of infection for rural areas

Rural area	No. of sample examined	No. of <i>T.saginata</i> egg positive cases	Percentage (%)
AL-Kerataa	326	1	0.3*
Senjar	454	2	0.4
Al-Wardea Kharej	290	0	0
Albo -Humaer	314	3	1*
AL-Seyahe	300	1	0.7
<b>Total</b>	<b>1684</b>	<b>7</b>	<b>0.41</b>

\* $P < 0.05$

Table 2 illustrates the percentage of infection for mentioned area according to the year of infections Where the highest infection rate (1.28, 0.78 and 0.93) % was for the Albo -Humaer region for the years (2018, 2019 and 2020) respectively, while in AL-Kerataa region their only one infection (1.06%) in 2019.

**Table 2:** The percentage of infection for rural areas according to the year of infections

Year	2018			2019			2020		
	No. of Cases Ex.	No. of <i>T.saginata</i> cases	%	No. of Cases Ex.	No. of <i>T.saginata</i> cases	%	No. of Cases Ex.	No. of <i>T.saginata</i> cases	%
AL-Kerataa	94	1	1.06	90	0	0	142	0	0
Albo -Humaer	78	1	1.28*	128	1	0.78	108	1	0.93*
Al-Wardea Kharej	93	0	0	65	0		132	0	0
Senjar	110	1	0.91*	96	0	0	248	1	0.40
AL-Seyahe	115	1	0.86	86	0	0	99	0	0
<b>Total</b>	<b>490</b>	<b>4</b>	<b>0.82</b>	<b>465</b>	<b>1</b>	<b>0.23</b>	<b>729</b>	<b>2</b>	<b>0.27</b>

\* $P < 0.05$

In the Table 3 showing the infection rate for male is (0.66%) higher than female (0.21%), and the results were statistically significant ( $p < 0.05$ ). A total of 5males (0.66%) out of the 753 screened male population were found infected with *T. saginata*, on the other hand, out of the 931screened females, 2 (0.21%) were found positive for *T. saginata* infection (Table 3).

**Table 3:** The percentage of infection according to the gender

Gender	No. of examined specimens	No. infection	%
Male	753	5	0.66
Female	931	2	0.21
<b>Total</b>	<b>1684</b>	<b>7</b>	<b>0.41</b>

Each fecal specimen reading for three replicates of the sample for Kato-Katz Method, and direct smears. Among 7 infection samples, 4 were positive for *T.saginata* eggs by direct smear method while 7 fecal specimen were positive by Kato-Katz Method, therefore, egg per gram (EPGs) of 7 people were calculated, and analyzed for the evaluation of correlations of EPGs among two stool examination methods, and the results were statistically significant ( $p < 0.05$ ), (Table 4).

As showing in Fig 1 The adult tape worm *T. saginata* (length: 1.80 m) expelled after the oral medications that are toxic to the adult tapeworm include Praziquantel (Biltricide) Albendazole (Albenza) for 4 years old female patient suffering pain, diarrhea, were morphologically diagnosis in the advanced parasites laboratory by dissecting microscope, the main uterine lateral braches in gravid proglottids were >15 in number suggesting that they are *T. saginata*.

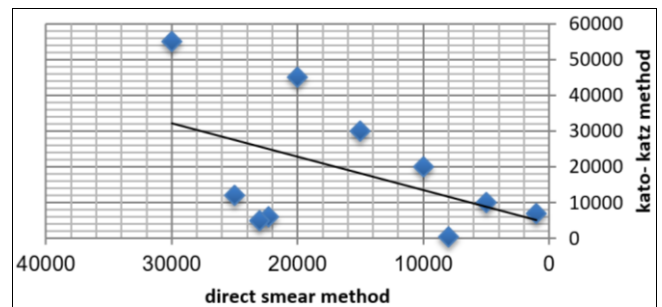
**Table 4:** The percentage of infection according to the method of diagnosis

Rural area	Gender	Age (Y)	Direct Smear Method	Kato-Katz Method	NO.of <i>T.saginata</i> cases
AL-Kerataa	F	4	-	+	1
Albo - Humaer	F	10	-	+	3
		12	+	+	
		14	+	+	
Al-wardea kharej	-	-	-	-	0
Senjar	M	54	-	+	2
		16	+	+	
AL-Seyahe	M	47	+	+	1
<b>Total</b>			<b>4</b>	<b>7</b>	<b>7</b>



**Fig 1:** The adult tape worm *T.saginata* (length of *T.saginata*: 1.80 m)

The (**Kato-Katz Method**) KK method and direct smear used for count numbers of eggs, and the range of egg counts was variable, and became wider when the EPG counts increased. The average egg per gram (EPGs) of 7 egg positive samples were 8700-58,488 by KK method, and 8000 -25,689 by direct smear method, (Fig 2).



**Fig 2:** Frequency distribution of eggs per gram of feces (EPG) determined by Kato-Katz method against EPGs by direct smear. A linear regression curve is plotted in the graph

In the studied areas, Table 5 shows the mean of hematological parameters in *T. saginata* infected and non-infected patients. The results showed a significant decrease in RBCs, Hb, and PCV ( $p \leq 0.05$ ). However, the results showed a marked increase in the WBCs, NUE and PLT for infected children compared to non-infected children.

**Table 5:** Comparative between hematological parameters in infected and Non-infected persons with *Taenia saginata* parasite

Hematological parameters	Infected patients M±SD	Non-infected persons M±SD
RBCs (X106/mm <sup>3</sup> )	5.49±0.56*	5.80±0.53*
Hb (g/dl)	11.90±1.8*	14.1±0.81*
PCV (%)	38.04±3.93*	39.62±2.65*
PLT (X10 <sup>3</sup> /mm <sup>3</sup> )	398.41±138.36*	354.84±55.13*
MCV (fL)	80.85±6.32	79.25±3.07
MCH (pg.)	28.15±4.67	26.79±1.9
MCHC (g/dl)	43.18±3.18	35.89 ±1.19
WBCs (cells/mm <sup>2</sup> )	15.11±5.09*	8.35±2.76*
Neutrophils (%)	76.35 ±20.16*	58.08±6.14*
Basophils (%)	1.5±0.32*	0.51±0.27*
Eosinophils (%)	5.09±3.41*	3.25 ±1.29*
Lymphocytes (%)	33.59±14.36	36.35 ±5.78
Monocytes (%)	5.38 ±4.73	5.37± 3.03
*Mean significant		

$P < 0.05$ \*

### Discussion

There are very few studies about tapeworms in Iraq in general and in Babylon Governorate in specific. Therefore, this study was conducted to find out the rates of infection with worms *T. saginata* in the current three years of research (2018-2020), and more studies are required to estimate its real infection rate. A few epidemiological studies estimate a prevalence of intestinal parasite infections in Hilla City, by Al - Kubaisi (2000) [1] where investigated 4537patients for hospitals of (Babil for childbirth and children hospital and Marjan Specialist hospital) and the results was 47.1% including *E coli* (10.9%), *E histolytica* (10.3%), *G. lamblia*(8.3%), *T hominis* (1.4%), *Chilomastix mesnili* (0.9%), *H nana*(2.2%), *Taenia saginata* (0.06%), *E vermicularis* (10.4%), *Trichuris trichiura* (1.6%), *A lumbricoides* (0.8%) and *A duodenale* (0.1%).This

prevalence tends to vary according to the population and its economic, infrastructure, education, culture and hygiene conditions, as well as to the presence of Cattle and livestock farms (Okello, and Thomas, 2017) [25].

Our results agree with study of Musa (2017) [24], in AL-Muthanna governorate showed the highest incidence rate of Taeniasis 5 patients while Wassit governorate showed (2 patients only). Study showed male participants have higher prevalence of infection (5) than female (2) patients.

There are many people in rural areas who suffering from parasitic infections due to poor sanitation, poor public health practices, increasing of vectors and malnutrition states in addition to using of river water directly for drinking and washing. In addition to the effect of the economic blockage in Iraq for long period leading to decreasing of drugs and sanitation

Not all human carriers of *T. saginata* infection will shed proglottids during the initial days of infection; it takes more than three months after infection with cysticercus larvae of *T. saginata* to manifest symptoms like shedding gravid proglottids either with feces or spontaneously. Only after this period of three months post-infection, will infected subjects test positive for Taenia eggs and proglottids in feces. Another problem possibly leading to an under-estimation of the prevalence, was that some people were unwilling to divulge their history of passing proglottids due to a self-assumed social stigma. (Iza *et al.*, 2020) [21].

Therefore, there is a good possibility that our sampling procedure might not have been wholly inclusive and some cases might have been left out, which suggests that the prevalence may be actually higher than reported in the current study and should There should be another investigative study for each rural area of the areas studied in our study.

The reason for this may be due to many factors, including the lack of environmental and health awareness among infected individuals, As well as the nutritional nature Educational level, cultural, social behavior and the psychological factor that has an effective effect in weakening the immune system by affecting the immune cells, but the most harmful factor is the coexistence of the individual with animals and the lack of adequate sanitary conditions.

Our results showing the infection rate for male is (0.66%) higher than female (0.21%), Table 3. The difference between the sexes can be due to the fact that males constantly eat beef meat, whether as a habit or like to eat meat or workers as male chefs who usually taste raw ground beef to obtain salt and spices, especially in the cafeterias and small restaurants spread in the streets and un cover and not authorized Private health institutions. Whereas, women do not fulfill these tasks and generally eat well-cooked food at home and become infected from time to time. Also, one of the important reasons is the consumption of partially roasted beef Therefore, eating meat by males in rural areas may contribute to an increase in the spread of infection among them. It has been observed in cafeterias where chefs add a piece of lard to the grill machine to create a scent and ambiance in the surrounding areas to attract customers. Second, vendors also apply a layer of grease to raw steaks while cooking to attract customers' attention. Thus, partially cystic larvae remain in undercooked beef. But it is quite important to keep in mind the beef-eating habits of the population sampled. However, further studies in Iraq's

provinces are recommended to actually prove that sex influences the prevalence of taeniasis.

Our results agree with (Eke *et al.*, 2014) [15] where in the prevalence of human taeniasis was more prevalent in males and attributed to the reason that males by habit eat more than females. The prevalence of *T. saginata* has been found higher in males (10.40%) than females (6.40%).

Also, taeniasis is prevalent in all regions of Thailand, except the South, infections were more frequently found in males than females of any age from 7-83 years. Almost double the number of infections was found in males (44) than in females (24). (Anantaphruti, 2013) [6].

According to our results Table 4 the youngest ages are more vulnerable to infection *T. saginata* for all regions of the study, maybe that due to these ages aren't understand the health standards of healthy food in the environment around them and eat undercooked beef.

The success and widespread distribution of this parasite can be associated with a range of factors including dietary habits (consumption of raw or undercooked cysticerci-infected meat), leisure activities in close proximity to grazing areas, free access of cattle to surface water, and sanitary education level of the farm workers as well as inadequate treatment and disposal of sewage In some territories, sewage channels are often open, and thus prone to flooding (Yassin *et al.*, 2006; Alhindi and Mervat, 2013) [36, 3]. This may increase the risk of animals coming into contact with pathogens in human sewage, such as *Taenia* eggs, and cattle or buffaloes contracting bovine cysticercosis (Alhindi and Mervat, 2013) [3]. Even in cases (e.g. in Tunisia) where sewage/wastewater is treated, *Taenia* spp. eggs could not be efficiently eliminated (Ben Ayed *et al.*, 2009) [8], whereas in some cases *Taenia* spp. eggs were even found in drinking water, such as in Iraq (Al-Morshidy and Al-Amari, 2015) [4]. Considering both the significant cattle and buffalo population, as well as the specific geographic, environmental and demographic characteristics of the area, efforts should be directed towards obtaining more detailed prevalence-based data by considering relevant aspects on the human, animal and ecosystem interface from a One-Health perspective. This would constitute the basis for the development of models predicting possible spatiotemporal transmission clusters and high-risk areas.

The lower prevalence of cysticercosis was found within the students, and it can be due to the access to education and basic infrastructure, as toilet, positive points on the protection for infection as previously reported (Holt *et al.*, 2016) [20], indicating that basic and general hygiene practices could diminish the infection levels in endemic areas. Another issue that could interfere with the knowledge or understanding of the importance of hygiene habits is the adhesion of the target population to health promotion initiatives (Sato *et al.*, 2018) [28].

The (Kato-Katz Method) KK method and direct smear used for count numbers of eggs and the range of egg counts was variable and became wider when the EPG counts increased. The average egg per gram (EPGs) of 7 egg positive samples were 8700-58,488 by KK method, and 8000 -25,689 by direct smear method (Fig 2). We conclude that KK method more qualitative and quantitative for diagnosis than direct smear method agree with the study of Beaver *et al.*, (1984) [7].

Many studies refer to the combination of KK method with direct smear resulted in increase of diagnostic

sensitivity. The “quick and thin” KK smear is easy to prepare specimen and make it cleared rapidly (15 min.), and the egg counts were proportional to those obtained by the classical KK smear (Peters *et al.*, 1980) [26]. A glass coverslip modification was also proposed to allow an immediate examination of a smear (Teesdale and Amin, 1976a, 1976b; Teesdale *et al.*, 1985 [32]).

From our results we concluded that both methods can be applicable for both qualitative and quantitative analysis in an endemic area.

However, this is not a limitation to this study; more elaborate and comprehensive epidemiological studies with a robust diagnosis and methodology, covering large areas of the population, must be planned in future to arrive at a firm conclusion about the actual percentage of the population harboring taeniasis due to *T. saginata* in Babylon province. Earlier studies of Wani *et al.* (2008); (2010) [34, 33] had reported a prevalence of 4.60%, 5.39% and 5.3% respectively from random populations in the Gurez region and district Shopian of the Kashmir valley; they attributed the infection to a low standard of living, poor sanitation, lack of personal hygiene, traditional agricultural methods, indiscriminate.

As human taeniasis is a zoonotic threat, with significant global prevalence and a large and underestimated impact on economy, agriculture and public health (Braae *et al.*, 2018) [10], more studies are required to estimate its real burden. Nevertheless, some epidemiological studies estimate a prevalence of taeniasis in Latin America between 0.24% and 17.25%. (Okello and Thomas, 2017) [25].

The decrease in certain hematological parameters (RBC, Hb and PCV) as a result of the colonization *T. saginata* in the digestive system, in particular duodenum, jejunum and iron absorption areas, result in bad absorption of sugars, fat, vitamins (like D and B12), folic acid, iron and zinc.

Iron deficiency results from poor iron absorption, which causes anemia because iron is incorporated into hemoglobin (Ehiaghe *et al.*, 2013) [14]. It is because of the difference in hematological parameters like the damage caused by each parasite species where worms generally have a greater impact than protozoa.

The increased number of certain types of white blood cells, particularly eosinophils, may be due to their role in the immune system's response to the treatment and elimination of intestinal parasites.

Eosinophilia is caused by the action of IL-5 produced by Th2 cells, the most important cytokine in the transformation is IL-5. It promotes the development of eosinophils and functions as an "eosinophil activator." One of the major reasons for the rise in parasitic diseases are characterized by the presence of eosinophils in the blood. Eosinophils act as effectors against parasites. The collection of parasitic Diseases are associated with a polarized Th2-type immune response, which is characterized by reactive cell types, eosinophils, neutrophils and mast cells (Al-Hasheme *et al.*, 2020) [2].

**Table 6:** Abbreviations

KK	Kato- Katz method
EPG	Eggs Per Gram
RBCs	Red blood cells
Hb	Haemoglobin
PCV	Packed Cell Volume
PLT	Platelets

MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
WBCs	White Blood Cells,
IL-5	Interleukin 5
TH2	T helper cell

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