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Intestinal Parasites of Experimental Rodents with Testing the Efficacy of Diagnostic Methods

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Abstract

From the beginning of January 2010 to the end of Jun 2011, three axes of parasitological investigations were performed in the animal house of Science College-Salahaddin University-Erbil/Iraq. In first axis, Twenty-Five adult albino rats were examined in which the overall prevalence with the different types of intestinal parasites was 76%. The following intestinal parasites were identified in the present study as: *Trichomonas muris* with the higher incidence of 56%, followed by *Scyphacia muris* 24%, *Giardia muris* 12%, *Hexamita muris* 8% and the least infection percentage was 4% for both *Entamoeba muris* and *Hymenolepis spp.* Single parasitic infection was the highest (52%), followed by double infection, 16%, and two cases of triple infection (8%). In the second axis of parasitological investigation: Ten adult albino rats and mice were selected for a comparison of diagnostic techniques by using direct and indirect (Zinc-Sulphate, saturated salt solution and sugar solution) floating methods for intestinal parasites existence. The most efficient method for detecting the infection rate with protozoa (*Entamoeba muris* 80% and *Giardia muris* 40%) in albino rats was by using saturated NaCl salt solution while in mice by using Zinc-Sulfate for *Entamoeba muris* 40% and *Giardia muris* 20%. The percentage of infection for both cestodes and nematodes in rats by using direct method were 50% and 30% and for mice, it was 30% and 40% respectively. The results of indirect methods for detecting these intestinal helminthes were the same when compared with direct method. In third axis, a comparison between using Scotch tape method and direct wet mount slide was carried out for the detection of *Syphacia muris* and *Syphacia obvelata*. Among thirty albino rats and ten mice examined, the rate of infection were 40% by using Scotch tape for both rats and mice while it was 16.6% and 30% by direct wet method respectively, showing that the former method (indirect) proved to be more efficient.

Keywords: *Intestinal Parasites, Rodents, Diagnostic Methods, Efficacy, Protozoans, Helminthes*

1. Introduction

The use of living laboratory animals as biological agents has provided knowledge to better understanding of both physiological and pathological processes in both man and other animals **(**Casebolt et al, 1988 and Dillehay et al, 1990).

Experimental results of research performed with living laboratory animals may be affected by physiological and immunological alterations caused by environmental conditions (Baker, 1998 and Weisbroth et al, 1998). The presence of infectious agents in animal house colonies represents a severe problem for biomedical research once murine parasitic agents protozoans and helminthes are known as the most frequent pathogens involved in immunological and metabolic alterations in the host (Pinto et al, 1994; Namue & Wongsawad, 1997 and Mahida, 2003).

In a study done in Brazil by Bicalho et al (2007), out of 344 laboratory mice and 111 laboratory rats were examined among different animal houses. Data have shown that the parasites revealed with their prevalence in the mice colonies were: *Syphacia obvelata* (92.3%); *Hymenolepis nana* (15.4%); *Spironucleus muris* (46.2%);

Giardia muris (46.2%); *Tritrichomonas muris* (53.8%); *Trichomonas minuta* (61.5%) and *Entamoeba muris* (84.6%), while in the rat colonies: *Syphacia muris* (46.2%); *Spironucleus muris* (85.7%); *Tritrichomonas muris* (85.7%); *Trichomonas minuta* (85.7%) and *Entamoeba muris* (85.7%). Pinto et al (2001) examined rats of different strains in the same country and found that they were parasitized with a nematode *Syphacia muris*, which is considered widely distributed and a very common nematode species occur in these hosts.

Gilioli et al (2000) carried our a parasitological study to determine the health status of 15 mouse and 10 rat colonies bred in 18 Brazilian laboratory animal houses. The prevalences of parasites detected among the investigated mice colonies were: *Syphacia obvelata* (86.6%); *Hymenolepis nana* (53.3%); *Giardia muris* (66.0%); *Entamoeba muris* (20.0%) and *Spironucleus muris* (80.0%) while in rats: *Syphacia muris* (80.0%); *Hymenolepis nana* (40.0%); *Spironucleus muris* (90.0%); *Giardia muris* (60.0%) and *Entamoeba muris* (80.0%).

From twelve wild rats trapped from wet Markets in Philippine, Claveria et al (2005) identified the endoparasites naming: *Hymenolepi diminuta*; *Moniliformis moniliformis*; *Taenia taeniaeformis; Capillaria hepatica*; *Trichosomoides crassicauda*; *Sarcocystis sp*. and two different species of stronglyloid-looking intestinal nematodes, and there were no recording of intestinal protozoa.

In Iraq, Hussein (1986) carried out the first isolation of *Giardia sp*. from Rattus norvegicus in Erbil city. Anyhow, in Iraq, relatively, few studies have been done on intestinal parasites of laboratory rats and mice, therefore, the objectives of the present study are divided in to three axes:

1. To survey the intestinal parasitic fauna of laboratory albino rats and mice.

2. To test the efficacy of diagnostic method for rodents intestinal parasites.

3. To compare *Syphacia muris* and *Syphacia obvelata* infection rate in both rats and mice using both direct and Scotch tape method.

2. Materials and Methods

During the beginning of January 2010 to Jun 2011, three different investigations were performed in animal house of Science College-Salahaddin University-Erbil/ Iraq:

1. Twenty-five adult albino rats (*Rattus norvegicus*) were anesthetized intraperitonially with ketamine hydrochloride (50 mg/kg) as reported by Maulood (2005). The gastrointestinal tract was separated from the animal, opened and their contents were then emptied into Petri dishes containing 0.85% physiological saline as stated by Belding (1965) and examined closely for the presence of intestinal parasites by using magnifying lens, dissecting microscope and multiple wet mount slides were prepared and examined by light microscope.

2. Stool samples of, ten each of adult albino rats and mice were examined looking for parasites by using direct wet mount slide and indirect floating technique method (Zinc-Sulfate, saturated salt solution and sugar solution) (Brown, 1969; Faust et al, 1978; Markell et al, 1999 and Al-Shirifi, 2000).

3. Thirty adult albino rats and ten mice were examined for detection of *Syphacia muris* and *Syphacia obvelata* by using Scotch tape method and compared with direct wet mount slide (Parija, 2004**)**. The detected parasites were identified according to their morphological characteristics and with the aid of Karen (2005) and with help of known reference books.

3. Results and Discussion

As shown in Table 1, the higher rate of prevalence, 56%, was reported for *Trichomonas muris*, is exactly in agreement with Bicalho et al (2007) but the lower rate, 4%, was observed for both *Entamoeba muris* and *Hymenolepis spp.*, which is not in agreement with the results of Bicalho et al (2007) and Gilioli et al (2000) for *Entamoeba muris*. The high rate of prevalence may be due to the living of rats in same plastic cage, dirty wooden chips and contamination of water and food.

Table 1. Prevalence of Intestinal Parasites Among 25 Examined Laboratory Albino Rats

Animals	Parasites	No. of Positive Rats	Prevalence %
Protozoa	*Giardia muris*	3	12 %
	Trichomonas muris	14	56 %
	Hexamita muris	2	8 %
	Entamoeba muris	1	4 %
Cestoda	*Hymenolepis* spp.	1	4 %
Nematoda	*Syphacia muris*	6	24 %

Table 2 shows that the overall rate of infection with different types of intestinal parasite was 76%, most of

infections were with 52% single parasite, followed by double parasite, 16%, in which flagellated intestinal protozoa *Trichomonas muris* observed with *Giardia muris*, *Hexamita muris*, *Syphacia muris* and *Hymenolepis spp.* Regarding triple infections there were two cases i.e. 1) 8% in which *Trichomonas muris* observed with *Entamoeba muris* and *Hexamita muris* 2) *Giardia muris* and *Syphacia muris*. *Trichomonas muris* and *Entamoeba muris* have been considered as commensal agents as they are not related to alterations of the animal health or interferences in experimental results. Sharp & La Regina (1998) declared *Giardia muris*, *Syphacia muris and Hexamita muris* as pathogenic agents.

Table 2. Single, Double and Triple Infection with Intestinal Parasites Among 25 Examined Laboratory Albino Rats

Types of Infection	No. of Positive Rats	%
Single		
Trichomonas muris	8	32 %
Giardia muris	1	4 %
Syphacia muris	4	16 %
Total Single	**13**	**52 %**
Double		
Trichomonas muris + *Giardia muris*	1	4 %
Trichomonas muris + *Hexamita muris*	1	4 %
Trichomonas muris + *Hymenolepis* spp.	1	4 %
Trichomonas muris + *Syphacia muris*	1	4 %
Total Double	**4**	**16 %**
Triple		
Trichomonas muris+ *Hexamita muris* + *Entamoeba muris*	1	4 %
Trichomonas muris+ *Giardia muris* + *Syphacia muris*	1	4 %
Total Triple	**2**	**8 %**
Total	**19**	**76 %**

Tables 3 and 4 show that there were differences among the methods, used for identification of intestinal parasites, revealed in percentage (%), from total number of 20 faecal samples (10 of each rats and mice) examined by direct microscopic examination. The rate of infection were 20%, 10%, 50% and 30% for *Entamoeba muris*, *Giardia muris*, *Hymenolepis* spp. and *Syphacia muris* respectively among rats, whereas the rate of infection among mice samples were 30%, 10%, 30%, 40% for *Entamoeba muris*, *Giardia muris*, *Hymenolepis spp.* and *Syphacia obvelata* respectively.

Table 3. Fecal Analysis for Detection of Intestinal Parasite of Albino Rats and Mice by Direct Method

Parasites	Direct Method			
	Rats(10)		Mice(10)	
	+ve	%	+ve	%
Entamoeba muris	2	20	3	30
Giardia muris	1	10	1	10
Hymenolepis spp.	5	50	3	30
Syphacia muris	3	30		
Syphacia. obvelata			4	40

The highest rate of infection, however, was with *Hymenolepis spp*. and *Syphacia obvelata* among rats and mice respectively, and the lower percentage of infection was with, *Giardia muris* among both groups. The high prevalence of *Hymenolepis spp*. and *Syphacia spp*. may be due to direct cycle *Hymenolepis nana*.

As the samples were positive by using both direct and indirect methods, therefore only five negative samples by direct method were compared with indirect method. The results of indirect methods for detecting intestinal helminthes parasites, (*Hymenolepis spp.*, *Syphacia muris and S. obvelata*) were the same when compared with direct method, while the best efficiency for the methods were reported by using saturated NaCl salt solution for detecting intestinal protozoa especially *Entamoeba muris*, 80%, and *Giardia muris*, 40%, in rats while in mice the best efficiency for the methods were reported by using Zinc-Sulphate for *Entamoeba muris* 40% and *Giardia muris* 20%.

Though, studies on comparison between direct and indirect faecal analysis methods on rats and mice are unavailable, however, most of the researchers concluded that Zinc-Sulphate is the best indirect flotation method for detecting intestinal parasites in human (Al-Kachache, 1989; Al-Daoody, 1998 and Ahmed, 2006).

Table 5 shows a comparison between Scotch tape and direct mount slide method for the detection of *Syphacia muris and Syphacia Obvelata* in rats and mice. The rates of infection with this parasite were higher i.e. 40% for both kinds of test animals, by using scotch tape method as compare to using direct mount slide method where the rates were 16.6% and 30% for rate and mice respectively.

Table 4. Faecal Analysis for Detection of Intestinal Parasites of Albino Rats and Mice by Indirect Method for Five Negative Samples by Direct Methods

Parasites	Indirect Methods											
	Zinc-Sulphate				Saturated Salt Solution				Sugar Solution			
	Rats (5)		Mice (5)		Rats (5)		Mice (5)		Rats (5)		Mice (5)	
	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
Entamoeba muris	2	40	2	40	4	80	1	20	3	60	1	20
Giardia muris	1	20	1	20	2	40	0	0	0	0	0	0

Table 5. Comparison Between Scotch Tape Method and Direct Mount Method for Detection of *Scyphasia muris* and *Syphacia obvelata*

Lab Animals	Scotch Tape Method			Direct Method		
	Examd. Animals	+ve	%	Examd. Animals	+ve	%
Rats	30	12	40	30	5	16.6
Mice	10	04	40	10	3	30.0

4. Conclusion

The results of the current study indicate the need of massive investment on laboratory animals: physical environment, equipments, human resources qualification, implementation of strict sanitary barriers and sanitary monitoring in the animal houses especially for biomedical research. Furthermore, quarantine programs are also needed so that new animals or biological materials can be isolated up to the moment their health status, may be assessed and then introduced into the sanitary-controlled colonies.

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