Neotrop. Helminthol., 8(2), 2014

ISSN: 2218-6425 impreso / ISSN: 1995-1043 on line

aphia
ASOCIACIÓN PERUANA DE HELMINTOLOGÍA E INVERTEBRADOS AFINES

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

OCCURRENCE OF GASTROINTESTINAL NEMATODES *ASPICULURIS TETRAPTERA (NITZSCH, 1821)* SCHULZ, 1927 AND *SYPHACIA OBVELATA* RUDOLPHI, 1802 ON *MUS MUSCULUS* LINNAEUS, 1758 FROM RESEARCH VIVARIA IN MEXICO

OCURRENCIA DE NEMATODOS GASTROINTESTINALES *ASPICULURIS TETRAPTERA* (NITZSCH, 1821) SCHULZ, 1927 Y *SYPHACIA OBVELATA* RUDOLPHI, 1802 EN *MUS MUSCULUS* LINNAEUS, 1758 EN BIOTERIOS DE INVESTIGACION EN MEXICO

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Suggested citation: Grano-Maldonado, M. 2014. Occurrence of gastrointestinal nematodes *Aspiculuris tetraptera* (Nitzsch, 1821) Schulz, 1927 and *Syphacia obvelata* Rudolphi, 1802 on *Mus musculus* Linnaeus, 1758 from research Vivaria in Mexico. Neotropical Helminthology, vol. 8, n°2, jul-dec, pp. 305-312.

Abstract

Laboratory mice *Mus musculus* Linnaeus, 1758 are commonly used as important models in veterinary and biomedical research. Forty laboratory mice were collected in four different vivaria at the National Autonomous University of Mexico and evaluated for parasites. Examination of intestinal organs revealed nematode *Aspiculuris tetraptera (Nitzsch, 1821) Schulz, 1927 (n=104) and Syphacia obvelata* Rudolphi, 1802 *(n=1582).* A statistical study was performed to determine host sex preference of infection. Cestode parasites, *Rodentolepis nana* (Siebold, 1852) synonymous *(Hymenolepis nana* and *Vampirolepis nana)* including a potential cause of human cestodiasis, with an emphasis on those pathogens with zoonotic potential. Evident ectoparasites were not present. A review reporting parasites on rodents employed on vivaria in Mexico was elaborated from a database at the National Helminths Collection of the Institute of Biology of the National Autonomous University of Mexico. This is the first report of the occurrence of these nematodes, *A. tetraptera* and *S. obvelata,* on *M. musculus* used and their known geographical distribution.

Keywords: *Aspiculuris tetraptera* - Mexico - *Mus musculus* - *Syphacia obvelata* - vivaria mice.

Resumen

El ratón común *Mus musculus* Linnaeus, 1758 es empleado comúnmente como modelo de investigación en las ciencias veterinarias y biomédicas. Cuarenta organismos fueron colectados en cuatro diferentes bioterios de la Universidad Nacional Autónoma de México y fue evaluada la presencia de parásitos. La examinación intestinal reveló al nematodo *Aspiculuris tetraptera* (Nitzsch, 1821) Schulz, 1927 (n=104) y *Syphacia obvelata* Rudolphi, 1802 (n=1582). El análisis estadístico determinó que no hay preferencia parasitaria por sexo del hospedero. Se registró al cestodo *Rodentolepis nana* (Siebold, 1852) sinónimos *(Hymenolepis nana* y *Vampirolepis nana)* que son causantes de cestodiasis en el humano. Este trabajo tiene un énfasis en estos helmintos debido a su potencial zoonótico. No se detectó la presencia de ectoparasitos. Se elaboró un reporte del registro de parásitos en roedores de bioterio en México obtenido de una base de datos de la Colección Nacional de Helmintos del Instituto de Biología de la Universidad Nacional Autónoma de México. Este es un primer registro preliminar de la ocurrencia del nematodo *A. tetraptera y S. obvelata* en el ratón común *M. musculus* en cuatro bioterios en la ciudad de México. Este trabajo amplía la distribución geográfica y contribuye también, a un nuevo registro del parásito.

Palabras clave: bioterios - *Aspiculuris tetraptera* - Mexico - *Mus musculus* - *Syphacia obvelata.*

INTRODUCTION

Mice are the most commonly used mammalian research model with hundreds of strains (Frasierand & Talka, 2005). They are primarily employed because they are mammals relatively easy to maintain and handle, reproduce rapidly, and share a high degree of homology in humans (Bronson *et al.*, 1989**;** CCAC, 1998). Proper housing and management of animal facilities are essential to i) provides animal well-being and maintain good health, ii) obtain quality of research data minimizing variations that can affect research results, iii) keep safe the health and safety of personnel (CCAC, 1998; Seward, 2001; Frasier & Talka, 2005; Pritt & Duffee, 2007). A parasitised animal may alter host physiology, making the host inappropriate for many experimental uses.

The most common rodent in house laboratory or vivaria is the common mice *Mus musculus* Linnaeus, 1758. However, many of pathogens of these laboratory mice may alter host physiology, making in some cases the mice host not fitting for many research works. Some prevalence of these pathogens may represent unwanted variables in research (Vogelweid, 1998; Parker *et al.*, 2009) performed an extended research report describing the infectious agents in laboratory mouse at University level in the same way, revealed the main infection centre may be wild rodents around laboratory facilities at the university. And monitoring these animals for parasitological infestations is important in order to exclude infectious agents requiring constant routine studies.

Several authors (Seward, 2001; Olfert & Godson, 2000; Roble *et al.*, 2012) underline the potential for laboratory animal personnel to serve as mechanical vectors of unwanted infective agents may increase when these persons handle infected mice at work or acquire self infected. The aim of the current study was to expand knowledge on the presence of helminth parasite and their taxonomical identification from laboratory mice kept on vivaria which are employed for different research studies. Data of abundance and the prevalence of parasites commonly found in populations of mice on the University campus in Mexico City are evaluated

and are discussed in the context of possible infectious disease outbreaks in campus vivaria.

MATERIAL AND METHODS

A total of 40 random picked up live mice were donated from four different vivaria. Live mice (20 males and 20 females) were transported in cardboard containers to the laboratory where they were euthanized using an overdose of anaesthetic ether solution. All animals were sacrifice in accordance with an approved institutional animal care ethical protocol (CCAC,1998). Mice were measured (mm) and total length (TL) registered. An evaluation for ectoparasites was performed under the stereomicroscope (LEICA MZ 9.5, Wetzlar, Germany).

For endoparasite examination, an abdominal dissection was performed using a sterile scalpel form the mouth thru the anus. The following major internal organs were collected: heart, lungs, pancreas, spleen, liver, kidneys, stomach, and intestine. Each organ was placed in individual Petri dishes with physiological saline solution 8.5% for further examination. Collections of nematodes were elaborated using small paintbrush placing the specimens on the saline solution. Fixation was elaborated using hot Berland liquid (19 glacial acetic acid parts and 1 formalin part) allowing the full body extension of the nematodes, after that each specimen were placed in vial with 70% alcohol to preserve specimens for further taxonomical examination. Nematodes were placed in slides with lactophenol of Amman (According with Lamothe-Argumedo, 1997) between cover slide and slide allowing the specimen to be transparent. Cephalic region and the extreme caudal of each specimen were cut using a scalp on a slide using drops of glycerine for better managing. All samples were evaluated microscopically using an optical microscope (LEICA DMLB 10 Wetzlar, Germany) and camera appliance for drawing taxonomic value features. Cestodes were collected and the cephalic region (scolex) were cut and put in a slide for further identification.

The taxonomic keys used were Yamaguti (1961), CIH key to the parasite nematodes (Anderson *et al.*, 1974-1983) and Keys to the cestode parasite of vertebrates (Khalil *et al.*, 1994). The prevalence was calculated by dividing the number of infected hosts with a particular parasite species by the total number of hosts of one species examined, expressed as a percentage. The average abundance was calculated by dividing the total number of individuals of a particular species of parasite by total number of hosts of one species examined (both infected and uninfected) of nematodes was determined according to the procedures of Margolis *et al.*, (1982). This study also sets out to examine the parasitic preference between male versus female parasitized mice using an x^2 test to assessment for an association between parasite infection and mice sex.

RESULTS

The examined specimens of *M. musculus* ranged in size from 370 to 510 (429 ± 53.47 mm) total length (TL). A total of 1686 nematodes were found in *Mus musculus* belonging to the *Aspiculuris tetraptera* (Oxyurida: Heteroxynematidae) and *Syphacia obvelata* (Oxyurida: Oxyuridae) in four vivaria (Table 1). These nematodes showed certain specificity for particular host organs and were found in the intestine only. One nematode *A. tetraptera* was found only in the mesentery, whereas the cestoda *Rodentolepis nana* (Siebold, 1852) were exclusive of intestine and cecum (Table 2). With reference to the mice's parasitism by the host sex, this study showed that there was no parasitic preference between the 40 parasitized mice; we established 20 females and 20 males ($c^2 = 0.023$, table 3.84; df = 0.1, alpha = 0.05).

DISCUSSION

This initial study was undertaken to identify parasites on four different vivaria on the mice *M. musculus* employed to different experimental

Table 1. Prevalence (%) and Abundance of nematode parasites on specimens of *Mus musculus* (n=40) collected at four different vivaria in Mexico.

Vivaria/ Faculty	*Aspiculuris* sp. (n= 104)	Prevalence	Abundance	*Syphacia* sp. (n= 1582)	Prevalence	Abundance
Science	0	0	0	196	60	39.2
Medicine	34	60	6.8	930	100	186
Veterinary	58	60	11.6	308	100	61.6
Bio medics	12	60	2.4	148	100	29.6
Mean	26	45	5.2	395.5	90	79.1
SD	25.56	30	5.11	362.50	20	72.51

Table 2. Helminths reported in rodents employed in house laboratories (vivaria) in Mexico. *(The National Helminths Collection of the Institute of Biology of the National Autonomous University of Mexico (CNHE-IBUNAM)).

Host	Parasite	Habitat	Geographical Location	Reference
Mus musculus	TREMATODA			
	Centrocestus formosanus	Intestine	Mexico City	Arizmendi-Espinosa (1989); (1992)
	Echinochasmus zubedakhaname	Intestine	Yucatan	Lamothe-Argumedo *et al.* (1991); Aguirre-Macedo (1989)
	Posthodiplostonum minimum	Intestine	Patzcuaro	Perez Ponce de Leon (1992), (1995); Aguirre-Macedo (1989)
	Phagicola angrensis	Intestine	Yucatan	Aguirre-Macedo (1989)
	NEMATODA			
	Aspiculuris tetraptera	Intestine	Mexico City	In the present study
	Syphacia obvelata	Intestine	Mexico City	In the present study
	CESTODA			
	Taenia taeniaeformis	Liver	Mexico City	Caballero (1938) (CNHE)*
	Vampirolepis nana	Intestine	Mexico City	García-Prieto (1986)
Rattus norvergicus	*TREMATODA*			
	Echinostoma ochoterenci	Intestine	Mexico City (Chapultepec)	Zerecero (1943)
	Echinostoma revolutum	Intestine	Cienega Lerma (Edo. Mexico)	Larios-Rodríguez (1940)

Continues table 2.

Continues table 2

	Fibricola caballeroi	Lungs	Mexico City	Larios-Rodríguez (1940);
	Paragonimus mexicanus			Ramirez (1986) (experimental)
	CESTODA			
	Taenia taeniaeformis (cists)	Intestine	Morelia, Mich.	Zerecero (1943)
	Vampirolepis nana	Intestine	Mexico City, Morelia, Mich.	Caballero (1939), (1942); Hierro-Huerta (1992)
	ACANTHOCEPHALA			
	Moniliformis moniliformis	Intestine	Morelia, Mich.	Hierro-Huerta (1992)
	NEMATODA			
	Capillaria sp	Bladder	Morelia, Mich.	Hierro-Huerta (1992)
	Heterakis spumosa	Intestine	Mexico city (Chapultepec)	Caballero (1939)
			Morelia, Mich.	Hierro-Huerta (1992)
	Gongylonema neoplasticum	Stomach	Morelia, Mich.	Hierro-Huerta (1992)
	Nipposstrongylus brasilensis	Intestine	Morelia, Mich.	Hierro-Huerta (1992)
	Trichuris muris	Intestine	Mexico city	Zerecero (1943)
Rattus rattus	TREMATODA			
	Paragonimus mexicanus	Lung	Mexico city	Ramírez (1986) (experimental)
	Schistosoma mansoni	Liver, spleen	Puerto Rico	No data registered
Rattus sp.	NEMATODA			
	Trichuris muris	Intestine	Mexico city	Osorio (CNHE)
	TREMATODA			
	Clonorchis sinensis	-	Japan	Donation, data no publish
	Paragonimus miyazakii	Lung	Japan	Donation, data no publish

biological, medical, biomedical and veterinary studies on the University campus. Helminthological survey results of rodents in this study showed intestinal parasites present in laboratory mouse colonies at the University campus in Mexico City were unknown and none documented by the Laboratory Animal Diagnostic employers. Researchers using mice, rats, and rabbits in biomedical experimentation should be aware of the profound effects that many of these agents can have on research (Baker, 1998). This author provides as extend research work concerning some pathogens and their direct effect on valid data research.

Experimental studies are required to evaluate the outline of infective agents in these mammals. Like all animals, even mice housed facilities are subject to infection (Olfert & Godson, 2000; Roble *et al.*, 2012), as well as mice sold as pets or feed other animals (Roble *et al.*, 2012). According with Hoag & Meier (1989) infections

with the various helminths rarely produce clinical signs and are only potentially important as producing unpredictable variables in animals used in research. Mouse colonies are often infected with the oxyurids (pinworms) *S. obvelata* and *A. tetraptera* (Parker *et al.*, 2009; Roble *et al.*, 2012) and in the latest study, other nematode parasites morphologically compatible with *Gongylonema* spp. were found in the gastrointestinal tract however was not found in the present study. Scott and Gibbs (1986) described the population dynamics of pinworms *S. obvelata* and *A. tetraptera* in mice. In Hidalgo state, Mexico, Pulido-Flores *et al. (*2005) reported these nematodes in wild rodents in, however, these authors reported that *Aspiculuris* sp. was the most intense and abundant. Allymehr *et al.* (2012) reported *S. obvelata* (prevalence 42%), *A. tetraptera* (prevalence 19%) in house mice from poultry houses in Iran. In the present study, *S. obvelata* was the most abundant agreeing with Allymehr *et al.* (2012), these results may be favoured by the vivaria conditions. These mouse pinworms have a direct life cycle and spread through a colony rapidly because of the large numbers of eggs excreted and carried by the wind. Some mouse colonies may be infected with *R. nana* (the dwarf tapeworm) (Parker *et al*., 2009; Roble *et al*., 2012). The life cycle of this parasite does not involve any secondary hosts, with infection taking place directly from eggs excreted in faeces. Heavily infected animals are below norms in weight and may be anaemic. Parker *et al.* (2009) conclude that wild rodents living near vivaria in some way transmit infections to and between the laboratory colonies and could serve as a source of infection or infestation in laboratory mouse colonies, although little is known about the prevalence of infectious diseases in wild mouse populations in Philadelphia in particular. Is well documentated, the wild mouse (*M. domesticus*) populations in Australia revealed a high prevalence of minute virus of mice (MVM), epizootic diarrhea of infant mice (EDIM), and Theiler mouse encephalomyelitis virus (TMEV), murine cytomegalovirus (MCMV*)*, as well as significant seroprevalence of mouse adenovirus (MAV), parvovirus, and thymic virus (murid herpesvirus 3) (Parker *et al.*, 2009). Further studies concerning virus and bacteria may be included during surveillance healthy programs in the university vivaria. Although the endoparasitic burdens found on this study were similar than other studies (most notably nematodes) (Parker *et al.*, 2009). Outbreaks infections present in laboratory mouse colonies in vivaria are well documented, despite improvement in detection these activities should be remain continuous for research institutions. However, their infection way is not always known.

The present study is an initial research which would assist further studies on the basic biology of the parasites and vivaria management. The helminth species reported in this studies were known to Parasitology discipline previously, but none had been reported in individual of *M. musculus* had been examined for parasites before this study research vivarium in Mexico City. This work showed the first approach to identify the helminth parasite from four different laboratory mice kept on vivaria which are employed for different research studies at University campus in Mexico City. *Hymenolepis* sp. can infect humans, and highlights the importance of a more robust need for parasite control in the vivaria facilities and increasing the number of other vivaria facilities in other universities merit further study. The employees may proceed to provide antihelmintic medication regularly in the context of possible infectious disease outbreaks.

ACKNOWLEDGMENTS

Thanks to Rafael Lamothe-Argumedo and Luis Garcia-Prieto at the National Helminths Collection of the Institute of Biology of the National Autonomous University of Mexico (CNHE-IBUNAM), for his help in acquiring specimens for taxonomical identification and literature. Special thanks to Fernando Garcia-Vargas for his editorial comments. This paper is dedicated to the late, Rafael Lamothe-Argumedo who sadly passed away during the writing of this manuscript.

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Received June 20, 2014.
Accepted September 22, 2014.