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STUDY ON THE ISOLATION AND IDENTIFICATION OF ASCARIS *LUMBRICOIDES* FROM SINDH, PAKISTAN

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Abstract

Ascaris lumbricoides is an intestinal parasitic nematode that causes the intestinal disease in animals. Due to its severe infection, their reproduction and production process becomes decreased. Therefore, the present study was conducted for isolation and identification of *Ascaris* from contaminated soil of 5 different locations namely two from SAU area, Jam Bungalows, Mir Colony and village Sultan Magsi, near Tandojam town .One hundred samples were collected from all the five locations (20 from each location) and contamination of soil samples were determined. The results revealed that out of 20 soil samples, each from Jam Bungalows and Mir Colony area of Tandojam where the 15 samples were found positive showing 75% contamination of soil by the *Ascaris* eggs while, 20 sample were collected from Animals farm at Department of Animal Reproduction (SAU) where the 11 found positive those showed 55% soil contamination of the *Ascaris* eggs. While, 20 samples collected from Livestock Experimental station Farm (SAU) and dry area of village Sultan Mangsi in which 9 were found positive those showed 45% with *Ascaris* eggs contamination. It was concluded that all the samples which were taken from each location were found to be contaminated with the density of *Ascaris* eggs contamination.

Introduction

Pakistan is an agricultural country, Livestock being sub sector of agriculture that plays a vital role in national economy of Pakistan (Sahito et al., 2012). It shares about 55% of agriculture, 11.5 to total GDP of the country. About 35 million rural populations are engaged in this sector (Sarwar et al., 2002). Leles et al., (2012) reported the original description and naming of *Ascaris lumbricoides* from humans by Linnaeus in 1758 and later of *Ascaris suum* from pigs by Goeze 1782, these species have been considered to be valid. *Ascaris* is a cyllinderical psedocoelomate nematode, *Ascaris lumbricoides*, an intestinal roundworm, which belong to the kingdom Animala, phylum Nematode, class Secernentea, sub-class Phasmideaa, order Ascaridida, family, *Ascarididae*, genus *Ascaris,* species *A. lumbricoides* (Maggenti and Nemata, 1991). Having highest prevalence in tropical and subtropical regionh, they are typically large worms (up to about 40cm long) the common name is a roundworm characterized by a mouth surrounded by three lips. Its body is a mixture of pink and white color. The eggs are eaten by the host but are usually ingested accidentally (the only exception being in the case of laboratory experiments in which doses are purposefully administered to subjects (Khuroo, 1996). Fertilization can occur, male nematodes uses chemotaxis to locate females, it uses Copulatory accessories such as papillae, spicules and its curved tail to direct sperm and stabilize the female during mating (Gaugler et al., 2004). The female produces as many as 200,000 eggs per day for a year. These fertilized eggs become infectious after 2 weeks in soil they can persist in soil for 10 years or more (Bethony et al., 2006). There are three forms of eggs, fertilized, decorticate and unfertilized. Thick, external mamillated layer is absent. Unfertilized eggs are larger (reaching 90µm in length) and more elongated in shape, being a mass of variably sized granules (Chong, 2003). Dangana et al., (2011) surveyed *Ascaris lumbricoides* was found among pupils of primary schools, four schools were also visited, total of 471 samples were collected. Only 1 (0.2%) pupil found was positive for *A. lumbricoides*. The prevalence of the infection among the age group showed that 1 (0.9%) in the age group (5 to 9) years were positive while, the sex distribution showed a prevalence of 1 (0.4%) among the male and no positive case was recorded among the females. Zheng et al., (2012) described that *A. lumbricoides* is the largest intestinal nematode parasite of man, which can lead to various

complications because of its mobility. As the esophagus is not normal habitat of *Ascaris*, the report of esophageal ascariasis is rare. Misra and Dwivedi, (2000) described the poor sanitation was the important risk factor for infection and women were more affected due to progesterone plays a role in inducing Oddi's sphincter relaxation, allowing the nematode to access the biliary duct. Although it was not common in developed countries, ascariasis infection was increasingly likely to be encountered by clinicians because of the growing rates of travel to developing countries and increased migration. Alba et al., (2009) described that the *A. lumbricoides* and *A. suum* manifested considerable similarity in protein profile.

Lumbricoides is a nematode parasite in the small intestine of various terrestrial mammals' chiefly herbivores. Mammals, from marsupials to human and non-human primates, birds, reptiles, fishes, cat and dog serve as common hosts. The dog's role is as a definitive host that reservoir hosts and biological transmitter of *Ascaris lumbricoides*. *A. lumbricoides* are environmental contaminators that increase risk of infection (Shalaby et al., 2010). The prevalence is greatest in areas where suboptimal sanitation practices lead to increased contamination of soil and water. Heavy infections with *Ascaris* are frequently believed to result in abdominal discomfort, pain, and diarrhea in animals. A mass of worms obstruct the bowel lumen, which is leading to acute intestinal obstruction. The obstruction occurs most commonly at the ileocecal valve. *Ascarisis* and *Trichuriasis* are the most intestinal helminthiasis transmission of disease occurs through ingestion or in halation of *ascaris* and *trichus* eggs embrocated in the soil (Arfaa, 2007). *A. lumbricoides*, infections in animals follows the ingestion of *Ascaris* eggs through contaminated foods or soil (Khuroo, 1996) which is found, eendemic in the Middle East and South America, especially in rural countries. Ascariasis infection causes about 20.000 deaths in every year. Further, Galzerano et al., (2010) reported that the *Ascaris lumbricoides* infestation is acquired through ingestion of eggs in raw vegetables. The human is the definitive host. Ingested larvae penetrate the intestinal lymphatic and venous vessels and through the portal vein reach the right heart, pulmonary circulation and the alveoli. In the bowel nematodes can perforate the intestinal wall, be ejected from the mouth or anus and penetrate the biliary ducts or the airways. Finally, Leles et al., (2012) conclude that *A. lumbricoides* and *A. suum* are a single species and that the name *A. lumbricoides* Linnaeus 1758 has taxonomic priority; therefore *A. suum* Goeze 1782 should be considered a synonym of *A. lumbricoides*. Evaluate the new origin and evaluation of *Ascaris* spp. in humans as well as in animals and the uniqueness of the species in both hosts genetically are same. Therefore, the study was continued to observe the *Ascaris* spp. from the egg sides, collected from moist and contaminated soil to check its eggs percent as well form the different locations of Sindh, province.

Materials and Methods

The present study was conducted to isolation and identification of *A. lumbricoides* through soil which increase *ascarisis* contamination in Tandojam, Hyderabad and its surrounding area. Tandojam is a rural town having Sindh Agriculture University with traditional agricultural type, consisting of rice, corn, maize, wheat, sugarcane, different vegetables and fruits, well cultivated soil and tropical in climate conditions. Most people are employed daily taking care of the crops, animals wastes are extensively used to enrich the soil by the indigenes.

The soil for isolation and identification of *A. lumbricoides* are collected from five different location ie., **(1)** Livestock Experimental Station Farm SAU- Tando jam, **(2)** Mir Colony Animals Farms, **(3)** Dry area of village Sultan Magsi, **(4)** Animals Farm Department of Animal Reproduction SAU- Tandojam and surrounding areas of **(5)** Jam Bungalows. The eggs of *Ascaris* spp. either *A. lumbricoides* or *A. suum* photographed under digital camera (10x) connected with CPU and monitor and showed as in figures in the results of the study.

A total of 100 soil samples were collected from five different areas of Tandojam, Hyderabad and its surrounding from shady and moist area to examine eggs of parasitic round worm (*Ascaris*) in soil. Soil packed and labeled in plastic bags. The samples were brought to the laboratory Department of Veterinary Parasitology, where each sample was subjected to homogeneous mixture and tested for *Ascaris* eggs according to the Flotation Technique. The samples were brought in to the laboratory and the each sample was tested for 3 times for observing the confirmation with eggs. This sample was kept in to the bottle which was covered with the cover slip at the edge of the bottle. But at the time of experiment, the cover slip was removed and kept under microscope to check the presence of *Ascaris* eggs. Finally, the presence of eggs was observed either the result was taken positively or negatively which showed the contamination with eggs on their different locations. The each and every one sample was transferred into properly marked microcentrifuge tubes, and kept in to a refrigerator for 20-25 min at 4-5^{o}C.

Results

During survey, 100 samples of soil were collected (20 from each location) to determine *Ascaris* eggs in soil. During the study it was observed, the out of 100 samples were also examined in which, 59% found positive soil samples, contaminated with *Ascaris* eggs. The results table-1 indicated that out of 20 soil samples, fifteen samples each from Jam Bungalows and Mir Colony area of Tandojam were found positive showing 75 percent contamination of soil by *Ascaris* eggs.

While, eleven positive soil samples out of 20 collected from Animals Farm Department of Animal Reproduction SAU- Tandojam area were showed 55 percent soil

Table 1: Showing prevalence percentage of *A. lumbricoides* during, 2012

S. No	Area	Sample size	No. Positive	%	Species
1	Jam Bungalows, Tandojam Mir Colony Farms	20	15	75	*A. lumbricoides*
2	Tandojam Animals Farm Department of Animals	20	15	75	*A. lumbricoides*
3	Reproduction SAU, Tandojam	20	11	55	*A. lumbricoides*
4	Live Stock Experimental Station SAU, Tandojam	20	09	45	*A. lumbricoides*
5	Dry Area of village Sultan Magsi, Tandojam	20	09	45	*A. lumbricoides*
Total	05	100	59	59 %	

Figure 1: A. lumbricoides which were taken from Jam Bungalows and Mir Colony

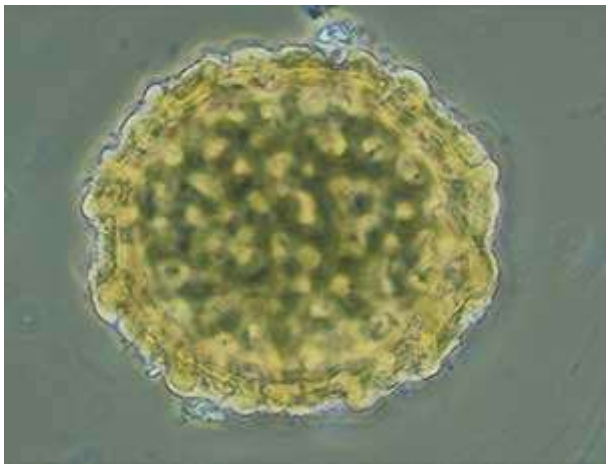
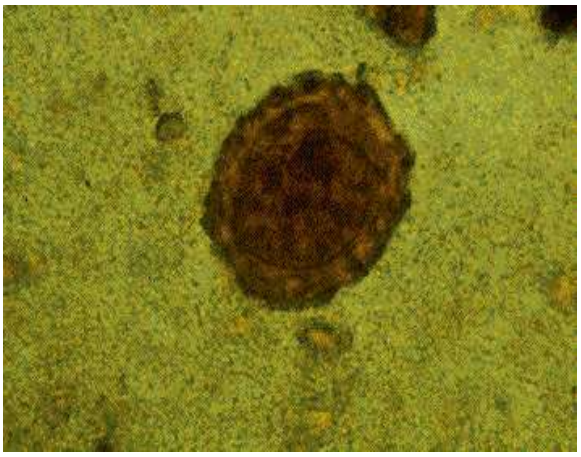


Figure 2: A. lumbricoides which were taken from Jam Bungalows and Mir Colony

contamination of *Ascaris* eggs, while nine soil samples out of 20 each collected from livestock

Figure 3: A. lumbricoides taken from Animals Farm of Department of Animal Reproduction SAU- Tando jam.



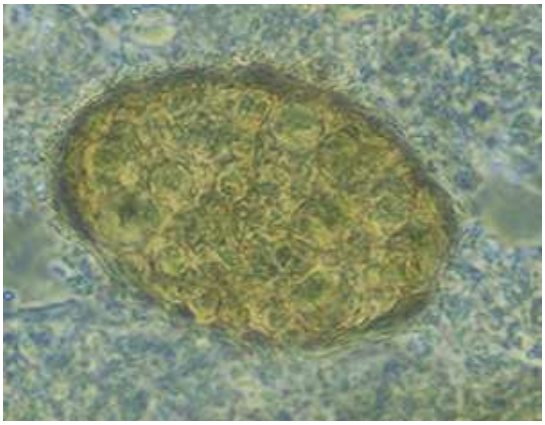
Experimental Station SAU- Tandojam and Dry area of Village Sultan Mangsi were found positive showing 45 percent *Ascaris* eggs contamination. The photos of the Figures 1 and 2 show the eggs of *A. lumbricoides* which were taken from Jam Bungalows and Mir Colony and Figures 3 and 4 show egg of samples which were taken from Animals Farm of Department of Animal Reproduction SAU- Tando jam. Then Figure 5 shows the eggs of samples which were taken the dry area of Village Sultan Magsi Tando jam and Livestock Experimental Station farm SAU- Tandojam.

Discussion

Large roundworm causes *Ascarisis* in small and large animals, also known as intestinal infection. *Ascaris suum* and *A. lumbricoides* are distributed worldwide and grow up to 40cm in length and these infections can be treated by use of *Ascaricides*. Infection of *Ascaris suum* occurs when its eggs, containing a third stage juvenile, are swallowed. The eggs are able to survive on their own for so long because they are resistant to strong chemicals, low temperatures and dehydration. *Ascaris* are considered as highly potential in terms of fecundity; they are probably the round worms of which eggs may be

Figure 4: A. lumbricoides taken from Animals Farm of Department of Animal Reproduction SAU- Tando jam.

Figure 5: A. lumbricoides taken the dry area of Village Sultan Magsi Tando jam and Livestock Experimental Station farm SAU- Tandojam.



found all over the earth and soil contaminated with *Ascaris* eggs is main source of infection. The study is agreed with the Galzerano et al., (2010) who reported that the eggs could be ingested from the raw vegetables and those can be hatched in to the abdomen ultimately the human is the definitive host of *A. lumbricoides*. The contradictory species study finally described by the Leles et al., (2012) who hypotheses relative to the confused to the origin species: 1. *A. lumbricoides* that infect to the human and *A. suum* that recorded mostly from pigs are both the valid species, with the two species originating via a speciation event from a common ancestor sometime before the domestication of pigs by humans, or 2) *A. lumbricoides* in humans is derived directly from the species *A. suum* found in pigs with *A. suum* then existing as a persistent ancestor after formation of *A. lumbricoides*, or 3) *A. suum* is derived directly from *A. lumbricoides* with the persistent ancestor being *A. lumbricoides* and *A. suum* being the newly derived species, and finally, 4) *A. lumbricoides* and A. suum are the same species, this hypothesis being supported by studies showing both low morphological and low genetic divergence at several genes.

The present study was carried out during the year, 2012 to see the soil contaminated with eggs of *Ascaris* and the level of contamination with *Ascaris* eggs. It is the etiological agent of *Ascarisis* which infects man. Humans become infected by ingesting infective eggs in contaminated food, water or from hands that have become facially contaminated. Following ingestion, the larvae hatch in the circulation where they are carried to the heart and lungs (Andrade et al., 2001). Sorenson et al., (1996) who reported that *A. lumbricoides* infection is common in children under the age of 10 years. The low prevalence of *A. lumbricoides* may be as a result of treatment of infected children who were screened by the previous researcher who had carried out the same work in the environment and probably the pupils must have been educated on the mode of infection and ways of preventing the infection hence very low prevalence was obtained in the survey.

The findings of the present study showed that out of 100 soil samples 59% gave overall prevalence of *A. lumbricoides* as shown in Table-1. These results are further supported by another studies like; Jarosz, (2001) who reported that out of 72 soil samples collected in public places of the city 13.9% were positive for *Ascaris* eggs and the mean egg density was 3.75/1 in soil. Laura et al., (2008) concluded that roundworms of the genus *Ascaris* are common parasites of the human gastrointestinal tract. Basam and Adnan, (2009) found that (72.9%) of examined individuals infected with different types of intestinal parasites and the highest Helminth detected were *Ascaris lumbricoides* (56%), followed by *Strongyloides stercoralis* (15.5%). Dangana et al., (2011) reported *A. lumbricoides* were surveyed among pupils of primary schools. Only 1 (0.2%) pupil found was positive for *A. lumbricoides* in the age group (5 to 9) years were positive while the sex distribution showed a prevalence of 1 (0.4%) among the male and sno positive case was recorded among the females.

Conclusion and Recommendation

It was concluded from the findings of the present study that the shady/moist areas/environment and the soils under buffalo herds established in Mir Colony and Jam Bungalows areas of Tandojam were with higher *Ascaris* eggs contamination as compared to soil samples of Animals Farm Department of Animal Reproduction, Livestock Experimental Station Farm (SAU) Tandojam and dry area of village Sultan Mangsi near Tandojam. Based on the findings and observation attention should be given to awareness of livestock farmers and agricultures on the mode of epidemiology, transmission of infection, control and treatment of the parasite *A. lumbricoides.* The origin of *Ascaris* spp. is still confused either *A. lumbricoides* and *A. suum* are truly distinct species or same.

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