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Soil Mediated Helminthiasis: A Study on Neglected Tropical Disease in Wild Animals in Haryana

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ABSTRACT

Background: Soil mediated/transmitted helminthiasis (STH/SMH) is a neglected tropical disease and includes Ascarids, *Trichuris* and Hookworms, which prominently affects population living either in the low sanitary condition or in close proximity with the animals. Recent years have witnessed elevation in the *Trichuris* infection, with India ranking first globally. Variety of factors including low sanitation, close contact with animals, increased man and wild conflict contribute towards STH. However, co-relation of STH/SMH infection from wildlife have not been studied.

Methods: The study was conducted for a period of 1 year from August, 2021 to September, 2022. *Trichuris* infection was present in 72 out of 131 carcasses of wild animals with prevalence rate of 35%. The prevalence percentage of *Trichuris* infection in different sub categories of wild animals was calculated. Seasonal prevalence rate was calculated with increased cases reported in summer season followed by monsoon. Additionally, fecal samples, worms and intestinal samples for further studies were collected.

Result: Wild animals act as bridge for transmission of various zoonotic diseases. However, zoonotic potential of parasitic diseases are neglected in wild animals with no systematic literature. Based on our study we illustrate that wild animals hold considerate potential plausibly demonstrating an *iceberg phenomenon.* Wildlife is an essential component in the epidemiology of many, if not most, zoonoses, wildlife should be taken into account in the risk analysis framework.

Key words: One-health, Soil mediated helminthiasis, *Trichuris*, Wild animals, Zoonoses.

INTRODUCTION

Soil-transmitted helminthiasis/soil mediated helminthiasis (STH/ SMH) is a neglected tropical disease subtype. Neglected tropical diseases (NTDs) are diseases which, despite being very common, do not receive much contemplation in impoverished or developing nations (Acharya, 2017; Hawdon, 2014). NTD's include 13 parasitic and bacterial infections including, Lymphatic Filariasis, Onchocerciasis, Dracunculiasis, Schistosomiasis, Chagas Disease, Human African Trypanosomiasis (HAT), Leishmaniasis, Buruli Ulcer, Leprosy, Trachoma and Helminth illnesses transmitted through soil (including ascariasis, hookworm infectionand trichuriasis. STH is a highly diverse group of parasites present across the globe causing chronic life-long infection (Dobson *et al.,* 2008).

Soil mediated helminthiasis affects population that depend largely on domestic animal species for their social and financial capital and have low levels of resilience (Jourdan, 2018; Lebu *et al.*, 2023). The threat of zoonoses is growing as a result of climate change, impact on biodiversity and the etiological agents' shifting hosts. Conflicts between humans and animals plays key element in the cycle of disease transmission (Ejezie and Akpan, 1992; Rodrigues *et al.*, 2018; Mitchell *et al.*, 2011; Jones, 2021). Moreover, with the recent emergence of perilous viral infectious diseases, certain parasitic disease transmission has been masked allowing them to multiply at a rate jeopardizing human and animal health at global level. Remote and rural areas including humans and livestock of low to middle income countries (LMIC), meagre sanitary condition, limited medical and veterinary services and poverty laid the founding rock of NTD's and most prominently STH (Matilla *et al.,* 2018; Hotez *et al.,* 2014). All aforementioned factors contribute to long-term effects in humans with ranging facets such as increased or sustained poverty due to deformity or other long-term sickness sequelae, stunted growth and development in children, unfavorable pregnancy outcomesand decreased productive capacity 12, more over, leading to modulation of the immune system and susceptibility to other pathogenic diseases and other diseases (Loukas *et al.,* 2021).

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Morbidity and reduced fitness associated with infection makes STH a major concern both for global health and for agriculture in endemic areas (Pullan *et al.,* 2014). The infectious stages of these parasites are abundant in the environment and, due to their robustness against environmental insult can persist for longer periods. Interspecies SMH transmission and transmission to humans have been reported in several studies (Phosuk *et al.,* 2018; Márquez-Navarro *et al.,* 2012; Dunn *et al.,* 2002). Lack of proper follow up with combine drug administration with water, sanitation and hygiene (WASH) interventions, hygiene education, community engagement and vaccines, diagnosis, reporting, awareness, policy barriers, *etc.* could be the anticipated cause of increased disease occurrence (Hotez and Damania, 2018).

SMH is important in human population transmission. Ascariasis, hook wormsand *Trichuris* species infection are all examples of SMH and are highly prevalent in India (Sack *et al.,* 2021; De Silva *et al.,* 2003). Estimated record of STH in humans records, 1.79 billion people (Bethony *et at.*, 2006; Keiser *et al.,* 2010) Opisthorchiasis in 11.2 million people 2007-2008, Schistosomiasis in 330 million in 2005 (WHO., 2017). STH infestation have commonly been observed affecting 1.65 billion people and 1.5 billion people in 2015 (Jorge and Poulin, 2018), respectively. Albeit, the infection in humans have been under surveillance and monitoring, recorded and traced, possible co-relation from wildanimals and zoonotic point of view remain uninvestigated.

According to 2016 report of Global Burden of Disease India ranked first in *Trichuris* infection reporting 68 million cases in human population also mentioning the potential of wild animals as reservoir. Subclinical stages of parasites favour transmission of infectious parasite directly to human population or through domestic animals *via* feco-oral route. As wild animals play role in zoonotic transmission, present study was conducted for estimation of prevalence rate of Trichuris infection in wild animals at Hisar, Haryana based on the Post-mortem Examiantion for a period of one year from August 2021-September 2022 to emphasis the role of wild animals in transmission of zoonotic parasites also raising global concern of *Trichuris* transmission from one-health point of view. The present manuscript deals with the recorded occurrence of *Trichuriasis* in wild animal species (ruminants) which plausibly play role in forming sylvatic cycle for STH/SMH transmission.STH or SMH is most profound category under neglected tropical disease. Also, zoonotic potential of the NTD is alarming as India ranks first in NTD occurrences.

Parasites that infect multiple host species are of particular concern because they are more likely to emerge than single-host parasites. A number of ecological and evolutionary factors influence the range of hosts that a parasite can infect. The diversity of parasites is directly related with the diversity of hosts. However, a recent study by Jorge and Poulin (2018), showed there is a weak association with host diversity and parasite discovery Sato *et al.* (2019). Our study was planned based on review studies, research articles, case reports and other relevant documented data available on STD/SMH at website of WHO, CDC articles published in various journals including NTD and NZD (neglected zoonotic diseases) related studies on PubMed, Research Scholar, Plos, Lancet global health, *etc*. The published review and research articles collectively present extensive data about *Trichuris* infection on human population with least coverage on Trichuris infection in animal population in India.Various reports on soil transmitted helminthiasis in humans are reported however the liaison of occurrence from animals as a source is not reported. Aforementioned SMH (Trichuris, hookworm and Ascarids), *Trichuris sp.* at present have been selected as the area of concern.

MATERIALS AND METHODS

The present study on SMH focuses on the *Trichuris* infestation in wild animal population at Haryana. The study was planned over span of one year (August 2021- September 2022) based on post mortem examination of wild animals received at Department of Veterinary Pathology, College of Veterinary and Animal Sciences, LUVAS, Hisar, Haryana. In particular months *Trichuris* worm load was quiet evident in wild animal carcass which took our interest to contribute further in this regard. The wild animal species comprises of Blackbuck, Chinkara, Cheetal, Sambhar, Peacock, Nilgai (Blue Bull) and a category of others including Monkeys, Porcupine and other wild birds which are less then frequently reported to LUVAS, Hisar. The present study was based upon the detailed examination of the carcass and collection of relevant samples from suspected cases (faeces, adult worms and intestinal scrapings). Also, as the study was based on post-mortem examination and thereby no ethical approval was needed.

Samples from the wild animals were collected with prior history of anemia, debilitated condition, pot-bellied appearance, tarry color feces and intestinal haemorrhages. Fecal samples were collected in sterile polybags for presence of parasitic eggs and identification. Intestinal scrapings were taken on clean- sterile slides and examined under microscope for presence of *Trichuris* eggs. Adult worms were collected from cecum region of the large intestine in sterile containers with 70% alcoholduring post-mortem examination for worm identification.

Post-mortem examination revealing intestinal haemorrhages in the submucosa were taken for histopathological evaluation. The pathological examination of the tissues was done by fixing them in 10% buffered formalin and sectioning at 4-5 microns and histopathological staining with H and E stain procedure. A total of 131 wild animals were examined during the study period. For the present study, the period of one year was divided into four seasons of summers, winter, autumn and spring. Statistical analysis of infection with Trichuris worms in wild animals were conducted to evaluate the prevalence rate of *Trichuris* in wild animals along

with information about the most affected species, months and season with highest rate of infection respectively. Estimation of prevalence rate for the study time was calculated using the formula:

Prevalence rate =

$$\frac{\text{No. of positive cases in particular species/months}}{\text{Total no.of cases in particular species/months}} \times 100$$

The data for present year was also compared with data obtained from previous year's post-mortem reports revealing deaths due to *Trichuris* infestation.

RESULTS AND DISCUSSION

The study on soil mediated helminthiasis for *Trichuris* infection in wild animals was conducted from August 2021-September 2022 with a total of 131 wild animals being

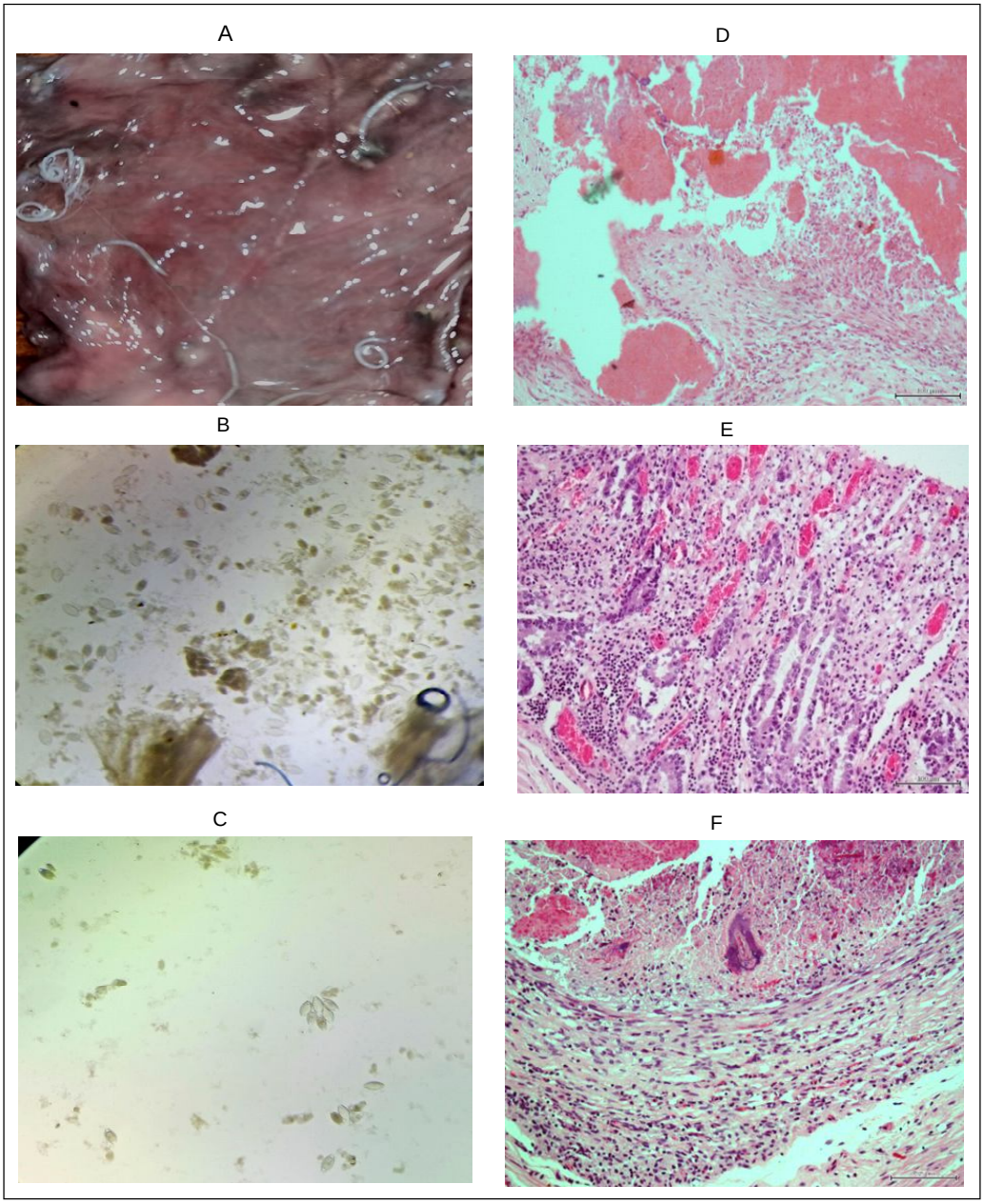

Fig 1: (A) Presence of Trichuris worm in the intestine. (B) and (C) Presence of Trichuris eggs as observed in the fecal materials under microscope 10X. (D) Photomicrograph of intestine showing platymyrian cells characteristic to nematodes (encircled). (E) Photomicrograpgh showing hemorrhages in the submucosal layer along with degeneration of the villi of intestine. (F) Photomicrograph showing presence of larvae surrounded by necrotic exudates in the intestine.

reported to the Department of Veterinary Pathology. Out of 131 wild animals Trichuris infection was confirmed in 72 animals.

Post-mortem examination

The animals were examined in detail with history of dullness, anemia, pale mucus membrane, pot-bellied condition. On examination of the visceral organs the intestine was filled with dark color or tarry coloured feces with cecum revealing presence of adult worm along with area of necrosis. In certain cases where in the adult worms were not recovered the fecal sample and intestinal scrapings were obtained for presence of eggs. Intestine showing reddeningof the serosal layer, hemorrhages in the submucosa were taken for histopathological studies. The intestines revealed haemorrhages (Fig 1E) various stages of the helminth parasite, including presence of larvae and parasitic eggs in the intestineas shown in figures (Fig 1D, 1F).

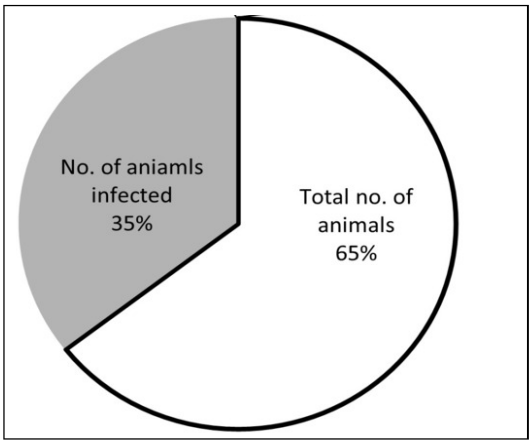

Fig 2: Wild animal reported during the study period of 1 year.

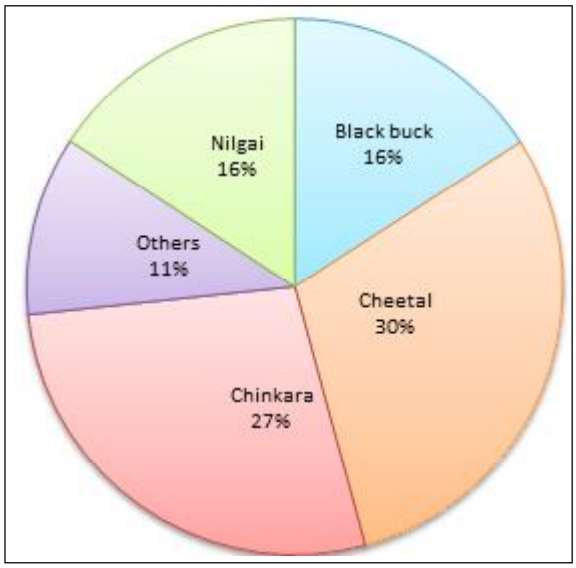

Fig 3: Total prevlance rate of *trichuris* sp. in diffrent wild animals.

Parasitological evaluation

The eggs of *Trichuris* sp. is easy to identify with unique dipolar appearance with presence of polar plugs on both the ends. Eggs containing larvae were also observed in the fecal samples. Eggs containing larvae are the source of transmission to others. Adult worms are white, 2-3 mm in size with long tails and henceforth they are known as whipworms. We used microscopy to confirm infection status by identifying Trichuris eggs. Thin smears of sedimented feces were examined under 10X objective magnification on a Olympus light microscope. Length, width, colorand contents of eggs were recorded and images were captured with a Nikon Camera (Fig 1B, 1C). Samples were considered positive for Trichuris when one or more eggs with the characteristic Trichuris ''lemon'' shape was identified as shown (Fig 1B, 1C) Samples were considered free of Trichuris only after the entire sediment was scanned and no Trichuris eggs were present.Reportedly, various species of Trichuris (*T. ovis*, *T. globulosa*, *T. skrjabini*, *T. discolor*) are known to infect ruminants and wild ruminants. However, identification of Trichuris species was not the prime focus of our study. Nevertheless,morphometric and molecular analysis in this regard could provide fruitful insights.

Prevalence estimation

131 wild animals were reported to the Department of Veterinary Pathology, LUVAS, Hisar. Out of these 72 wild animals were found to be positive for Trichuris infection. The infectifivity rate accounted for 35% infection of *Trichuris* over the study period as represented in pie chart in Fig 2. During the study period the wild animal species reported includes Blackbuck (29) with 16 positive cases, Cheetal (9) with 9 positive cases, Chinkara (14) with 12 positive cases, Peacock (22) with 8 positive cases, Nilgai (51) with 27 positive cases, sambhar (2) none positive and 4 in other categories with no reports of *Trichuris* infection as represented in Fig 4. Trichuris infection in various wild animals showing prevalence percentage of 16%, 30%, 27%, 16% and 11% in Black-buck, Cheetal, Chinkara, Nilgai and other animals respectively is represented in pie-diagram (Fig 3). Additionally, month-wise prevalence of Trichuris infection in respective months of October, November, December, January, February, March, April, May, June, July, August, September was observed as 72%, 60%, 53.84%, 48.40%, 50%, 100%, 50%, 75%, 60%, 41.67%, 46.15% and 53.33% respectively with higher prevalence rate during summer season (corresponding to months from March-May) represented in Fig 5, which are in accordance with other similar studies conducted in animals. The present data of infectivity was compared with previous years infectivity data of year 2019-2020 and 2020-2021.

The comparison showed increase in rate of *Trichuris* infection from previous yearsas represented in Fig 6. However, the increment rate was high for the present year. Prevalance rate of Trichuris infection of year 2021-2022 was compared with that of 2019-2021 in all wild animals. Trichuris prevalence for 2019-2020 and 2020-2021 was

25% and 28 % respectively, corresponding to 35% infectivity in 2021-2022.

Globally, STH has widespread affections in human populations majorly affecting the Asian countries especially south Asian countries including India. Substantial studies in human context in India have been conducted emphasizing STH's zoonotic potential linking animals to human transmission (Sato *et al.,* 2109; Labo *et al.,* 2011; Salam and Azam, 2017). In the present study, wild animals' carcasses dealt revealed Trichuris infection with an estimated overall prevalence of 35%. Although, comparativeto previous studies, Trichuris prevalence in our study was found to be elevated. Similar preliminary study on wild ruminants were conducted by Sengar *et al.* (2017) reporting

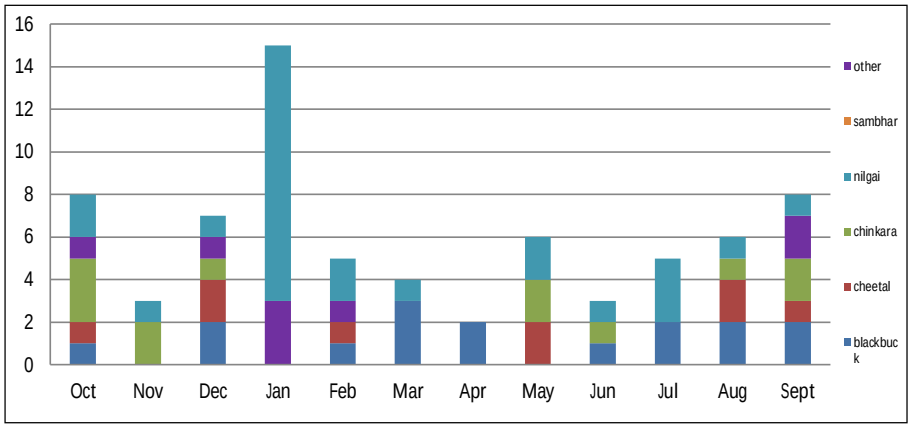

Fig 4: Number of animals having *Trichuris* infestation during the study period.

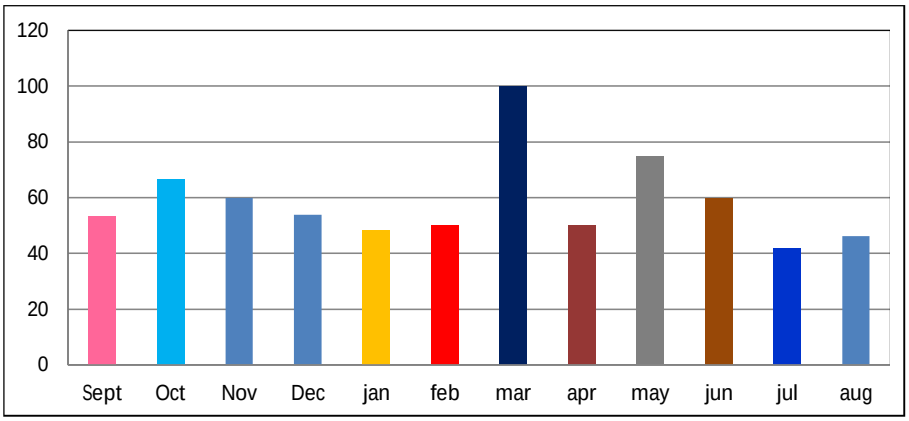

Fig 5: Month wise prevelance rate of *Trichuris* species infestation.

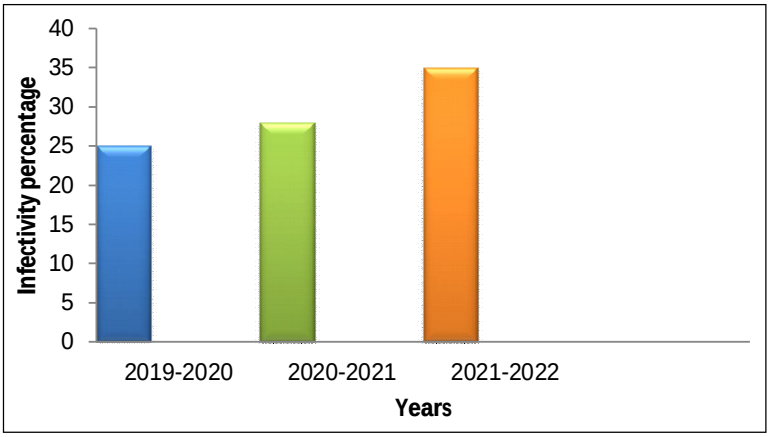

Fig 6: Comparison of infectivitey precentage with previous year.

11% and 7% in wild animals and domestic animals respectively 30 while Snehil *et al.* (2018) reported 5% (2/40) *Trichuris* infection in Deer and 11.76% infection in nilgai (2/17) Vohra *et al.* (2020). Recent studies by (Vohra *et al.,* 2020; Kalkal and Vohra, 2021) conducted on free ranging animals in Haryana for *Trichuris* infection was reported to be about 46%, 48% and 22% respectively in sheep, goats and pig population hinting a possible transmission of infection from wild to domestic animals (Vohra, 2021; Tebano *et al.,* 2023). The data compiled in present study was also divided on seasonal occurrence of *Trichuris* in animal population, revealing highest prevalence in summer season (March-May) followed by spring, monsoon and winter which was in alignment with other studies conducted.

CONCLUSION

Conflicts with wild animals are increasing because of decrease forest cover and increased urbanization. In remote areas, where animals are mostly free-ranging, have immense outlet for contracting infection from wild. Zoonotic diseases accounts for about 75%ofemerging infectious disease and can be devastating to both human and animal health globally. Many reports have shown that disease burden caused by SMH was underestimated due to lack of proper diagnostics in developing and under-developed countries. SMH transmission or Parasitic diseases are neglected in wild animals with no systematic and statistic literature. SMH in wild is major health hazard and holds zoonotic potential. “One Health” strategy should be implemented as it will not only help in diagnosing and preventing diseases, but would also result in improving the animal and human health. The primary concern is that there is critical gap between public health needs and veterinary responsibilities.

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Conflict of interest

The authors report no conflict of interest.

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