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DIVERSITY OF PROTOZOAN PARASITE AND BACTERIAL SPECIES ON THE EXTERNAL BODY SURFACE OF HOUSEFLIES COLLECTED FROM TWO DIFFERENT LOCATIONS OF KHANDESH REGION

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ABSTRACT:
House flies are closely linked to microorganisms during each stage of development. It can transmit pathogens through contaminated legs or mouthparts, and regurgitation while feeding. The primary intension of the present study was to identify the diversity of protozoanal and bacterial species on the external body surface of houseflies. For six months, from April 2022 to September 2022, the adult Musca domestica were collected from Amalner and Dhule cities, a total of 120 and 149 flies, respectively, were collected. The parasites and bacteria adhered to the external body surface of the individual flies were then vigorously shaken in sterile saline solution (5ml) for 2 minutes. After that, the solution was put into a test tube filled with nutritional broth and cultured for 6 to 8 hours at 370C. Three protozoan parasites (Giardia intestinalis, Entamoeba histolytica, and Balantidium coli) and one helminth parasite (Ascaris lumbricoides) were isolated from these flies. These results suggest that houseflies should be taken into account as potential mechanical vectors of human intestinal parasites in the present research area.

Keywords:- Houseflies, Ascaris lumbricoides, Giardia intestinalis, mechanical vectors, parasites, pathogens

INTRODUCTION :

Musca domestica L. the common house fly (Diptera: Muscidae), among other household pest is commensal insect that can endure in a variety of situations. It can frequently inhabits varieties of habitat like homes, food markets, and also in close proximity to human beings as well as domesticated animals and animal farms, garbage, human waste and feces (Malik, 2007). Adult houseflies dwell around human and animal settings as well as dump yards for food and breeding. It can consume variety of food like animal excrement, sweat, waste, and wet or decomposing pet waste and also consume milk, sugar, beef broth, and any other materials found in populated regions. They may breed in a variety of climates but prefer warmer (ideally 30°C) and drier.

House flies are intimately linked to microorganisms during each stage of development (larvae, pupae, adults) (Nayduch, 2017). It can transmit pathogens through contaminated legs or mouthparts, and regurgitation while feeding (Sasaki, 2000, Onwugamba, 2005). Even when food and oviposition sites are in ample supply, they travel an average distance of a few hundred metres to several kilometres in a couple of days (Winpisinger, 2005, Nazni, 2005).

Furthermore, some microorganisms that are momentarily linked with the flies can persist for days in the flies' mouthparts and crop [Sasaki, 2000]. This indicates the capacity of these flies transportation of pathogens over great distances between various habitats. Over 100 potentially antibiotic-resistant pathogenic bacteria, fungi, parasites, and even viruses have been discovered in or on M. domestica adults and larvae, according to a recent analysis (Onwugamba, 2005). The number of pathogens

carried by M. domestica varies according on the region where it is gathered and includes over 100 bacteria, fungi, viruses, and parasites.

Houseflies caught from human habitation and livestock farms were particularly evaluated for the presence of pathogens (Awache and Farouk 2016). Given that the number of pathogens in the stomach is typically higher than the number on the body's surfaces, feces and vomitus may also play a significant role in the spread of diseases.

The present study aimed to identify the bacterial and protozoan parasite species isolated from external body surface of houseflies collected from two different locations i.e. Dhule and Amalner cities of Khandesh region.

MATERIALS AND METHODS:

Sample collection :

The adult Musca domestica were collected from two different cities viz. Amalner and Dhule for six months i.e. from April 2022 to September, 2022. A total of 120 and 149 flies were collected from both the cities respectively. Animal farm house, hospitals and homes were chosen for the collection of the flies. A total of 59 houseflies collected from three animal farms, 21 from two hospitals and 40 from four homes from Amalner city. Flies were collected into sterile 50ml Falcon tubes and immediately transported to the lab. Based on morphology sexes of flies were identified. Individual flies then shaken vigorously in sterile saline solution (5ml) for 2 minutes to dislodge the parasites and bacteria adhered to external body surface (Fotedar, 2001). The solution then transferred in a test tube containing nutrient broth and incubated for 6-8 hours at 37oC for culture (Fotedar, 2001).

Isolation and identification of bacteria:

Eosin methylene blue (EMB) agar, mannitol salt (MS) agar, and xylose lysine deoxycholate (XLD) agar (all from HiMedia, Mumbai, India) were used for inoculating the culture through a sterile loop. After 24-48 hours of incubation at 37°C, colonies with a black centre in XLD medium, a golden yellow core in MS media, and a metallic sheen in EMB agar were identified as Salmonella spp., S. aureus, and E. coli (Bergey, et al., 1974). To verify morphological identification, Gram's staining technique was utilised (Cheesbrough, 1985).

RESULTS:

Flies diversity:

A stereomicroscope was used to properly identify houseflies with an identification key. A total of 120 and 149 flies were collected from Amalner and Dhule cities respectively. Of the total flies collected from Amalner city, 78 were Musca domestica, 28 were Musca autumnalis, 14 Chrysomya megacephala. Similarly these data for Musca domestica, Musca autumnalis and Chrysomya megacephala were 89, 37, and 23 respectively for Dhule city. Table 1 shows the distribution of flies in both the cities and number of male and female flies.

Isolation and identification of parasites

Of the total flies collected from Amalner city (120 flies), 69 (57.5%) were found to be parasitized with various parasites and their cysts (Table 1). Three protozoan parasites and one helminth parasite were identified from these infected flies (Table 2). Out of all the parasitized flies, 43% had Giardia intestinalis infestations, followed by Entamoeba histolytica (38%), and Balantidium coli (27%). Similar to this, out of total flies collected from Dhule city (149 flies), 81 (54.4%) were found to be positive for various parasites. Among these positive flies 37% had Entamoeba histolytica, 34% Balantidium coli and 25% had Giardia intestinalis infestations. Ascaris lumbricoides infestation for flies from both the cities were 8% and 9% respectively.

Isolation and identification of bacteria:

Each fly was washed with 1ml of phosphate-buffered saline by gently vortexing for 40s. After

washing the solution was used as an "external sample." A 100ul aliquot of the fly homogenate was used for bacterial isolation. The number of homogenates from animal setting were (n=34), and (n=51) from Amalner and Dhule cities respectively. Similarly these numbers for hospital setting were (n=27) and (n=31) and for dump yard were (n=59) and (n=67) for both the cities.

Three bacterial species were identified and these are 17 isolates of S. aureus, 15 isolates of Salmonella spp., and 4 isolates of E. coli were obtained from Amalner City and the number for S. aureus was 21, Salmonella spp., 19 and 2 isolates of E. coli were obtained from Dhule City. The largest percentage of bacteria were isolated from houseflies collected from poultry and animal farm (>65%), whereas the lowest percentage were obtained from household flies (<28%).

DISCUSSION:

According to Kassari et al. (2012), Graczyk et al. (2002) and Graczyk et al. (2005), the eco-biology of the housefly makes it a potential mechanical vector for the transmission of a wide variety of animal and human bacteria, viruses, and fungi. Since houseflies pick up diseases through their mouths, legs, wings, and other body parts during the feeding process and then transmit the pathogen back to the animal or person, where they complete their life cycle, several bacterial species have been isolated from the external surface of houseflies (Khamesipour, et al., 2018).

A variety of intricate aspects influence an organism's microbiome. For insects, the microbiota is primarily regulated by developmental stage, physiochemical conditions in various gut compartments, sources that are easily accessible for acquiring microbes (such food and habitat), and transmission of germs to progeny. This study is the first of its kind to examine the bacterial communities connected to M. domestica using samples taken from various places. As a result, the information provides not only a thorough understanding of the microbial variety but also information about the impact of the habitat. Specifically for the bacterial component of the exterior microbiota, the results show that house flies have an extremely diversified microbiota that is impacted by habitat and geographical origin.

The external surface of the house flies carried a distinct bacterial strains in contrast to the interior microbiota, particularly when the sample area has a lot of bacterial sources, like the farm habitat. In the current investigation, three bacterial species Salmonella spp., E. coli, and S. aurues were isolated. Additionally, it was discovered that every fly was a carrier of at least one parasite or bacterial spp., most likely as a result of poor hygiene and sanitation in the environment from where they were collected. It is consistent with a recent study that found Shigella, E. coli, Salmonella Typhimurium, and S. aureus on the outer surfaces of houseflies that were circulating in a university canteen in Dhaka, Bangladesh (Parvez, et al., 2016).

Houseflies were collected for this investigation from three separate places: hospitals, poultry and livestock farms, and residential areas from two different cities. Hospitals, poultry farms, and other places where animals are kept are particularly prone to mutations and antibiotic resistance. Most bacterial species have been found in poultry and livestock farms. This is consistent with a study from Brazil by Almeida, et al. (2014). Staphylococcus spp., Salmonella spp., and E. coli were found on the interior and external surfaces of houseflies collected from dairy farms, according to the study by Almeida et al. (2014). Hospital regions also have the highest amount of bacteria that have been found. The recent findings are similar to those of Nazari et al., who discovered that flies around a hospital in Hamadan, Iran, contained more

bacterial strains overall. Ommi, et al. (2016) reported that the amount of Salmonella and Campylobacter spp. recovered from houseflies collected from livestock farms, animal hospitals, and slaughterhouses was significantly higher than that from the home kitchen.

The previous study and the current one are related because different bacterial species, including E. coli, Staphylococcus aureus, Staphylococcus albus, Pseudomonas aeruginosa, Klebsiella, and Salmonella, were isolated from houseflies collected from human and animal settings (Yalli, et al., 2017). According to all of these investigations, houseflies have the capacity to transmit these harmful bacteria to people and animals through their external body.

In this study three protozoan parasites were isolated from external body surface of houseflies from various location of two cities (Amalner & Dhule). A protozoan that was commonly noted, Giardia intestinalis, suggests that it is an important human intestinal parasite. Entamoeba histolytica and Balantidium coli were the second and third most often isolated protozoans, respectively. Early studies also discovered these parasites on the body surfaces of houseflies and cockroaches (Tatfeng et al., 2004).

CONCLUSION:

According to the current analysis, house flies in the present study region may harbour human intestinal parasites as well as pathogenic bacteria. Identification of intestinal parasites that are present on external surface of houseflies in the area under research and could be dangerous to humans, especially young children. This suggests that the sanitary and hygienic situation needs to be improved right now. Additionally, to prevent food, utensils and water contamination, insect proofing equipment should be used in restaurants, kitchens, sweet shops, and slaughterhouses. The current study also acts as an epidemiological tool to track the prevalence of intestinal parasites and the current state of sanitation in the study area. To properly comprehend the relevance of harmful pathogens carried by flies, continuous surveillance is required. Flies from hospital environments carry pathogenic organisms that must be controlled by a hospital authority following suitable administrative processes. To prevent flies from entering the kitchen, a fly net should be used to stop their entry. Proper disposal of animal and human waste, as well as other decomposing objects, is necessary to stop multiplication of filth flies.

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Table 1

Flies	Amalner (120)		Dhule (149)		P value
	Male	Female	Male	Female	
Musca domestica	36	42	40	49	0.03775
Musca autumnalis	11	17	15	22	
Chrysomya megacephala	8	6	10	13	

Table 2: Prevalence of protozoan and helminth parasite on the houseflies collected from both the cities

Parasites	Amalner City (120)				Dhule City (149)			
Protozoans	**Animal Farm (63)**	**Hospitals (28)**	**House hold (29)**	**Total**	**Animal Farm (84)**	**Hospitals (31)**	**House hold (34)**	**Total**
Giardia intestinalis	18	05	06	29 (43%)	11	06	05	22 (25%)
Entamoeba histolytica	12	07	07	26 (38%)	19	07	06	32 (37%)
Balantidium coli	09	04	06	19 (27%)	17	03	08	28 (34%)
Helminths								
Ascaris lumbricoides	4	-	1	5 (8%)	5	-	2	7 (9%)