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Isolation of *Escherichia coli* and *Salmonella* species in flies trapped at animal farm premises

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Abstract
Present study was designed to explore the types of flies prevalent at food animal farms and their role in harboring the bacterial species namely *Escherichia coli* and *Salmonella.* Flies were trapped using indigenous traps from the cattle, sheep, goat, poultry farms and veterinary clinical complex. A total of 2187 flies were collected from the 36 pools during the months of April and May, 2019. All the collected flies were identified as house flies (*Musca domestica)* except one were identified as a blow fly (*Calliphora erythrocephala*). Processing of representative fly pool samples for isolation and identification of bacterial pathogens revealed 100% prevalence of *E. coli* in all the flies caught, whereas *Salmonella* was isolated from only 21 pools with a prevalence rate of 58.33%. It is thus concluded that flies prevalent at food animal farms are dominated by *Musca domestica* species and their role in harboring and transmission of *E. coli* and *Salmonella* species cannot be ruled out.

Keywords: Prevalence, *E. coli, Salmonella* species, flies, food animal premise

1. Introduction
Flies are very ubiquitous in nature and generally attracted towards human and animal dwellings for feeding and breeding. Millions of fly species are in existence and they must have typical ecological niche as per their nesting and feeding preferences. Flies associated with food animals and poultry constitute a major group of vectors. They could play potential role in disease transmission to humans, especially for foodborne pathogens [1, 2]. Flies collected from hospital premises were found to harbor antimicrobial resistant bacteria of human significance [3]. Flies are considered as an important transmitter of pathogens of public health significance and flies like housefly, stable fly and blow flies could harbor more than hundred species of bacterial pathogens [4, 5]. A recent study showed that flies can carry multidrug-resistant bacteria belonging to specific clonal lineages identical to those found in animal manure [6]. Flies are mechanical vectors and able to carry pathogenic bacteria from varied environmental sources [7]. In an environment flies have adopted free habit for feeding on food and organic matter like feces and dung. They can carry microbes on their body and even inside the gut and while feeding on human food or through direct contact, they transmit the pathogens [8]. Studies on the detection of bacteria in flies captured from food animal farms are not undertaken in our context; hence attempts were made to explore the types and role of flies in harboring ubiquitous important organisms namely *E. coli* and *Salmonella* species.

2. Materials and Methods
2.1 Sampling
Flies were trapped from the food animal farm premises in and around Shirwal and Veterinary Clinical Complex of this institute using traditional traps prepared indigenously. The unconventional fly-traps were made out of plastic water bottles. Dried fish and chicken powders in the ratio of 2:1, mixed with sugar syrup were used as a bait to attract flies inside the trap. Traps were fixed at different places inside the farms of cattle, sheep, goat, and poultry (Fig 1). Site selection was predetermined while making observations for the abundance of flies. For each food animal species, trapping was attempted six times; i.e. six pools of samples per species were collected. A detail of the fly collection is mentioned in table 1. Using no specific selection criteria, 40 percent of each catch was processed, provided that the number of flies caught was not less than 15, in that case all the flies were taken into processing and bacterial isolation.

Table 1: Details of flies trapped at animal farm premises

Sr. No.	Site	No. of trappings	Actual flies trapped per sampling						Total flies
1	Cattle farm	6	31	25	18	40	71	49	234
2	Sheep farm	6	47	39	44	53	50	48	281
3	Goat farm	6	75	72	73	85	87	98	490
4	Pig farm	6	40	30	112	62	75	91	410
5	Broiler farm	6	45	22	96	62	124	97	446
6	TVCC	6	16	74	48	62	55	71	326
7	Total	36	254	262	391	364	462	424	2187

Fig 1: Fly collection from various food animal farms using the indigenously made fly trap

2.2 Identification of fly species and isolation of bacterial pathogens

Flies were first washed with 1x Phosphate Buffer Saline (PBS) to initiate exterior washing. Following this the genus identification was done using microscopic view (10 x) of the wing pattern as per the method described previously with the use of vinyl gloves, sterilized petri dishes and toothpicks [9]. Identified flies were pooled and washing was carried out three times with 1x PBS to reduce surface contaminants, as per the method of Zhang and colleagues [10]. Washed fly samples were transferred to the centrifuge tubes having sterile PBS, crushed with sterile metal rod and centrifuged for 10 mins at 12000 rpm. The solid debris gets settled at the bottom and supernatant was again centrifuged for 10 min at 6000 rpm. The supernatant obtained after this process was used for bacterial isolation.

Isolation of *E. coli* from all the samples was done in accordance with the standard methods of Bergey's Manual of Systemic Bacteriology and OIE Terrestrial Manual [11, 12]. For the isolation and identification of *Salmonella* spp. standard procedures mentioned in FDA Bacteriological Analytical Manual were followed [13]. Rehydrated culture media specific for both *E. coli* and *Salmonella* species procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India was used for enrichment and selective plating of the organisms. For the pure culture isolation, enrichment followed by selective plating on specific agar medium was performed. Enrichment of fly samples was done in Enterobacteriaceae Enrichment Broth (EEB). Briefly, 1 ml centrifuged supernatant was inoculated in 5 ml of EEB and incubated at 37 °C for 24 hr. A loop full of this culture was used for selective plating carried out on the Eosin Methylene Blue (EMB) agar and the plates were incubated at 37 °C for 24 hr. Similarly, for isolation of pure culture of *Salmonella* species, samples were processed for pre-enrichment, enrichment and selective plating. Pre-enrichment was carried out in the Buffered Peptone Water (BPW). One ml of the centrifuged supernatant from fly samples was inoculated in 9 ml of BPW and incubated at 37 °C for 24 hr. For enrichment, 1 ml of the pre-enriched culture was inoculated in 5 ml of EEB and incubated overnight at 37 °C 16-18 hr. A loop full of the enriched culture was used for selective plating on the Xylose Lysine Deoxycholate (XLD) agar plates, which were further incubated at 37 °C for 24 hr. Five representative colonies from EMB agar and XLD agar plates were further subcultured and bacterial confirmation was attempted by colony characteristics, staining, morphology and biochemical tests namely, IMViC, catalase, oxidase and H_2S production.

3. Results and Discussion

From each animal farm premise, fly trapping was done six times, thus a total of 2187 flies were collected from the 36 pool samples during the course of study. Identification of captured flies revealed all the flies belonging to *Musca domestica* species which is also known as a house flies. Other than house fly, we could trap single fly identified as *Calliphora erythrocephala* which is also known as blow fly in Veterinary Clinical Complex of the institute. These findings are indicative of the fact that *Musca domestica* is predominant inhabitant of the food animal farm premise. Abundance of blood sucking stable fly and house fly in the dairy cattle farm was noticed in a study from Czech Republic [14]. A study from

Thailand has also indicated the maximum abundance of houseflies followed by blow flies and flesh flies in the urban areas and animal farms [15]. (Fukuda *et al.*, 2018). In this study trapping was performed during summer months (March-May) indicating the abundance of *Musca domestica* during these months at the study area. *Musca domestica* is the most widespread and commonly distributed fly species globally. They are abundant in the premises of hospitals, food markets, livestock and poultry farms. They are responsible for creating nuisance to humans and animals. *Musca domestica* is also responsible for carrying microbial pathogens which may cause serious illness in humans and animals. Molecular analysis revealed that house flies carry different groups of microorganisms [16].

Bacteriological studies revealed isolation of *E. coli* from all the fly pool samples trapped from all sources, whereas, *Salmonella* species could be isolated from 21 pools with a prevalence rate of 58.33 percent. *E. coli* showed typical green metallic sheen on the EMB agar while *Salmonella* species exhibited opaque colonies with black centers on the XLD agar (Fig 2 & 3). Flies are considered as important transmitters of pathogens of public health significance and flies like housefly, stable fly and blow flies could harbor more than hundred species of bacterial pathogens [4, 5]. Flies are considered as mechanical vectors for transmission of zoonotic bacterial pathogens and there is possibility that frequent contact of flies with animals and their fecal matter could acquire pathogens in their gut as well as external body surfaces. Their feeding, breeding and nesting habits make flies a potential reservoir and vector for transmission of pathogens and antimicrobial resistance traits [4, 5]. The house flies are able to reproduce in decaying organic matter of animal farms and are found to live in close association with animals. Potential of flies as a vector for foodborne pathogens like *E. coli*, *Staphylococcus aureus* and *Listeria monocytogenes* trapped from kitchen and animal proximity sites was conducted by Barreiro and colleagues [17]. Their findings revealed significantly higher prevalence *E. coli* in the flies collected from animal proximity sites. A study on *Musca domestica* released into rooms containing hens challenged with *Salmonella enterica* serovar Enteritidis revealed rapid contamination of flies with the Salmonella. About 40-50% flies were contaminated with Salmonella within 48 hr [18]. Study on microbiota of house flies collected from ten dairy farms throughout Denmark revealed dominant microbiota of phyla Firmicutes, Fusobacteria, Bacteroidetes, Protobacteria and Actinobacteria [19]. A potential of flies from swine farm in transmission of Salmonella species was also reported from Taiwan [20].

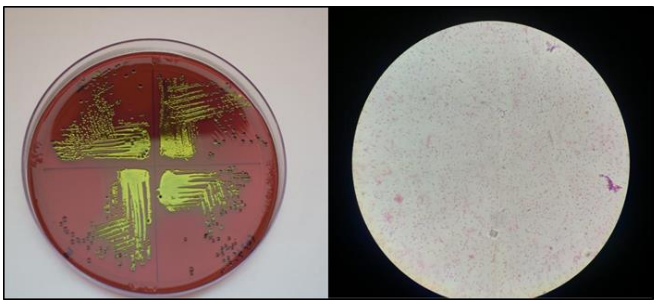

Fig 2: Typical metallic sheen of *E. coli* on EMB agar and *E. coli* stained as gram negative pink rods

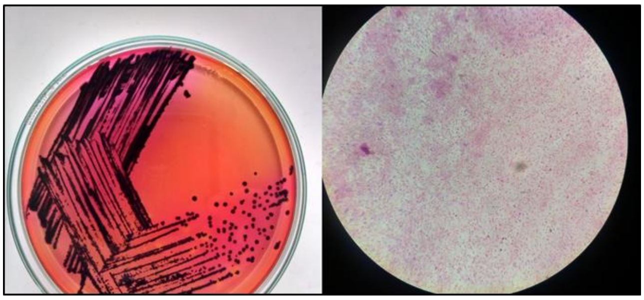

Fig 3: Black centered colonies of *Salmonella* spp. on XLD agar and *Salmonella* stained as gram negative pink rods

4. Conclusion

From the present study it can be concluded that flies inhabitant of the food animal farm premises predominantly belong to *Musca domestica* species. These flies harbor *Escherichia coli* and *Salmonella* species inside their crop and gut which emphases on their potential role in transmission of foodborne zoonotic pathogens.

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6. References

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