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OCCURRENCE OF SOME ZOONOTIC MICROORGANISMS IN FAECAL MATTER OF HOUSE RAT (*RATTUS RATTUS*) AND HOUSE MOUSE (*MUS MUSCULUS*) TRAPPED FROM VARIOUS STRUCTURES

M. MUSHTAQ-UL-HASSAN, I. HUSSAIN[1], B. SHEHZADI, M. SHAHEEN, M. S. MAHMOOD[1], A. RAFIQUE[2] AND M. MAHMOOD-UL-HASSAN[3]

Department of Zoology, GC University, Faisalabad; [1]Department of Microbiology, and [2]Department of Zoology and Fisheries, University of Agriculture, Faisalabad; [3]Department of Ecology and Wildlife, University of Veterinary and Animal Sciences, Lahore, Pakistan

ABSTRACT

Commensal as well as wild rats and mice may present a potential risk to public health. They may harbour microorganisms that can be transmitted either through contact with infected rodent urine or faeces, or through ectoparasites. Prevalence of zoonotic microorganism in house rat (*Rattus rattus)* and house mouse (*Mus musculus)* in different types of structures such as grocery shops, sweet shops, rice godowns, wheat godowns and poultry feed stores was studied in Sargodha and Faisalabad districts of Pakistan. In *R. rattus*, *E. coli* showed the highest prevalenc in faecal material from rice godowns (4.72%), while the lowest prevalence (3.46%) was in poultry feed stores (P<0.05). *S. typhi* had the higest prevalence (4.44%) in samples from grocery shops and lowest (3.56%) in those from sweet shops (P<0.05). Similarly, the highest prevalence of *H. nana* (3.89%) was recorded in faeces from grocery shops and lowest in those from poultry feed stores (P<0.05). In case of *Mus musculus*, rats from sweet shops showed the highest prevalence of *E. coli* (4.83%), while the lowest was in rats from wheat godowns (4.05%; P<0.05). *S. typhi* had the highest prevalence (4.56%) in grocery shops and lowest (3.28%) in sweet shops (P<0.05). For *H. nana*, the highest prevalence (3.61%) was noted in samples from rice godowns and lowest (3.00%) in sweet shops (P<0.05). Fecal samples of *R. rattus* and *Mus musculus* of all the structures of Sargodha district were significantly more infected with *E. coli*, *S. typhi* and *H. nana* compared with the samples collected from the structures of district Faisalabad (P<0.05). These results indicated that both house rat and house mouse may act as reservoir for *E. coli, S. typhi* and *H. nana.*

Key words: House rat, house mouse, *E. coli*, *S. typhi, H. nana*, faecal sample.

INTRODUCTION

Rats and mice play a significant role in public health, chiefly due to their role as carriers or reservoirs for microorganisms associated with infections and diseases that can be transmitted to humans. These diseases include plague, salmonellosis, leptospirosis, murine typhus, rickettsial pox, lymphocytic chorio-meningitis, rat-bite fever, hanta virus haemorrhagic pulmonary syndrome, haemorrhagic fever, Venezuelan equine encephalitis *(Alphavinus),* powassan encephalitis (*Flavivinus*), rabies, Rocky Mountain spotted fever and tularemia as well as parasitism such as trichinosis, eosinophilic meningitis, taeniasis, crypto-sporidia (Singleton *et al*., 2003) and *Trypanosoma lewisi* (Linardi and Botelho, 2002). Transmission of these infections to humans occurs by indirect contact. Some are transmitted through contact with infected rodent urine or faeces, others through fleas and lice, and still others through mosquito bites (Ruiz, 2004). According to Battersby *et al*. (2002), rural wild brown rats on farm serve as vectors of zoonotic and many other diseases and may represent a serious risk to the health of human and domestic animals. Rats are found to be infected with a number of zoonotic parasites including cryptosporidium, pasturella, listeria, yersinia and Hantavirus, and represent a potential risk to the health of humans and domestic animals.

The brown rats *(Rattus norvagicus)* from Doha, Qatar have been reported to be infested by *Hymenolopis diminuta* (Abu-Madi *et al.*, 2001). Salmonella in faecal pellets of wild brown rats *(Rattus norvagicus)* in the west Midlands is sufficient to present a potential risk to public health (Hilton *et al*., 2002). The present work was undertaken to isolate and make a preliminary investigation to zoonotic bacteria and helminths from house rat (*R. rattus)* and house mouse (*Mus musculus)* from different structures and poultry feed stores.

MATERIALS AND METHODS

Collection of the samples

Five structures *i.e*., grocery shops, sweet shops, rice godowns, wheat godowns and poultry feed stores from Faisalabad and Sargodha districts of Pakistan were sampled. Ten samples per collection, comprising

10 pellets of faecal matter of each of the house rat (*R. rattus*) and house mouse (*Mus musculus*) were taken from the structures. The sampling was done fortnightly for a period of eight months *i.e*. from March through October, 2005. Each faecal sample was placed in 8 to 10 ml phosphate-buffered-saline (PBS) in a plastic bottle and stored in a refrigerator till processed.

Isolation of microorganisms

Each sample was processed for the presence of bacteria and helminths following the methods described by Battersby *et al.* (2002). Briefly, two methods *i.e*. direct smear method and salt floatation technique were applied to find out the helminth eggs/larvae. For direct smear method, a drop of faecal sample thoroughly mixed with normal saline was placed on a slide and a cover slip was placed over the smear. Two slides of each sample were observed under the microscope at 40X.

Salt floatation solution was prepared by adding NaCl in distilled water upto saturation level. Faecal matter was thoroughly mixed in this solution. After straining, the solution was centrifuged for 5 minutes at 1500 rpm. After this, a few drops from centrifuged solution were placed on slides and examined under microscope and eggs and larvae were identified following Hayat and Akhtar (1999).

For bacterial isolation, media selective for *S. typhi* and *E. coli* were prepared as described by Cappucino and Sherman (1996). *Salmonella shigella* agar (SS Agar) was prepared for *S. typhi* and MacConkey's agar was used for *E. coli* following Yaqoob *et al*. (2007). Each sample was inoculated on the Petri's plates of each media, incubated at 37°C overnight and suspected colonies were re-streaked to obtain pure culture (Cruickshank *et al*., 1975).

Identification and characterization of isolates

Plates were observed for characteristic shape, color and other features of colonies for *Salmonella typhi* and *Escherichia coli* (Athar *et al*., 2007). Slides were prepared from discrete colonies, stained by Gram's staining method and observed under oil immersion lens of the microscope at 100X for examination of shape, arrangement and staining reaction of isolates. Methyl red test, indole test, nitrate reduction test, Voges-Prauskaur's (VP) test, urease test and hydrogen sulphide (H_2S) production test were biochemical tests adopted. In sugar fermentation reaction, xylose, glucose, lactose, sucrose, maltose, arabinose, raffinose, inositol and mannitol solutions were used.

Statistical analysis

Positive samples for *E. coli, S. typhi* and helminths were recorded from the faecal matters of *R. rattus* and *M. musculus* collected from poultry feed stores and other structures under study. Studentized t-test was applied for data analysis.

RESULTS

In the present study, maximum faecal samples from *R. rattus* were found infected with *E. coli* from rice godowns (4.72%), followed by grocery shops (4.22%; Table 1). The prevalence of house mouse infected with *E. coli* showed non-significant difference between grocery shops, sweet shops and rice godowns. Rice godowns showed higher prevalence than for sweet shops, wheat godowns and poultry feed stores, differences among the latter three structures were non significant (Table 1). Faecal pellets from the house rats of grocery shops infected with *S. typhi* (4.44%) were significantly higher ($p<0.05$) than sweet shops and wheat godowns, whereas the faecal samples collected from *R. rattus* of rice godowns and poultry feed stores showed non significant difference with all other structures under study. The prevalence of *H. nana* in grocery shops and rice godowns was significantly higher ($p<0.01$) than sweets shops and poultry feed stores, whereas the same in wheat godowns showed non significant difference with other structures (Table 1).

For *Mus musculus*, prevalence of *E. coli* was significantly higher in the faecal pellets collected from grocery shops, sweet shops and rice godowns than poultry feed stores and wheat godowns, the difference between the latter two structures was non significant. Faecal pellets collected from house mouse (*Mus musculus*) of grocery shops and rice godowns revealed non significant difference from each other in relation to the percent prevalence of *S. typhi* but both of these had significantly higher prevalence than from *Mus musculus* of sweet shops, wheat godowns and poultry feed stores (Table 1). Faecal pellets of *Mus musculus* collected from grocery shops and rice godowns were significantly higher in *H. nana* prevalence than sweet shops, whereas the samples from wheat godowns and poultry feed stores had non significantly different prevalence of *H. nana* compared to all other structures (Table 1).

DISCUSSION

The prevalence of *E. coli*, *S. typhi* and *H. nana* in the faecal matter of *R. rattus* and *Mus musculus* was studied in the present manuscript. *E. coli* are a type of faecal coliform bacteria found in sewerage, contaminated food and water. Rats and mice mostly live in house sewerage pathways or animal farms from where they can pick *E. coli* by eating contaminated feed, drinking water, wastes through poultry and cattle excreta and also from infected herbage. *E. coli* in the present study remained the most prevalent bacteria

Table 1: Percent prevalence of *E. coli, S. typhi* and *H. nana* from the faecal samples of *R. rattus* and *Mus musculus* from different structures of Pakistan

Structure	*R. rattus*			*Mus musculus*		
	E. coli	*S. typhi*	*H. nana*	*E. coli*	*S. typhi*	*H. nana*
Grocery shops	4.22ab	4.44a	3.89a	4.39a	4.56a	3.56a
Sweet shops	3.56b	3.56b	3.00b	4.83a	3.28b	3.00b
Rice godowns	4.72a	3.89ab	3.83a	4.56a	4.33a	3.61a
Wheat godowns	3.72b	3.61b	3.28ab	4.05b	3.56b	3.33ab
Poultry feed stores	3.46b	3.91ab	2.97b	4.12b	3.56b	3.33ab

Values within a column with different letters are significantly different ($p \leq 0.05$) from each other.

Table 2: Percent prevalence of *E. coli, S. typhi* and *H. nana* isolated from faecal matters of *R. rattus* and *Mus musculus* from two districts of Pakistan

Locality	*R. rattus*			*Mus musculus*		
	E. coli	*S. typhi*	*H. nana*	*E. coli*	*S. typhi*	*H. nana*
Faisalabad	3.14b	2.79b	3.00b	3.69b	3.36b	3.13b
Sargodha	4.97a	4.78a	4.00a	4.72a	4.50a	3.61a

Values within a column with different letters are significantly different ($p \leq 0.05$) from each other.

isolated and identified form the faecal matter of *R. rattus* and *Mus musculus*, which is in agreement with Cizek *et al.* (1999), who reported that 5.3% of rodents stool samples were found positive for *E. coli* in Czech Republic.

S. typhi are a group of bacteria that can cause diarrheal illness (salmonellosis) in humans. They are usually transmitted to rats and mice through eating contaminated feed and wastes of infected human, animals, raw food of animal origin, contaminated water, milk and sewerage wastes (Hilton *et al.*, 2002).

In the present study, the prevalence of *S. typhi* from different structures ranged from 3.28 to 4.56%. A previous study revealed that 5.1% mice and 16.2% rats were positive for Salmonella spp (Henzler and Optiz, 1992). During another study in UK, 4.91% of the rats and mice were found to carry Salmonella infection (Healing, 1991). Hilton *et al.* (2002) studied the prevalence of Salmonella in Midlands UK by culturing faecal dropping (n=100) and rectal swabs (n=50) of Norway rats and reported that 8% of faecal samples and 10% of rectal swabs were positive for Salmonella, which are in close agreement to the results presented in the present study.

Among the helminth parasites, *H. nana* is one of the most common rat tapeworms. In the present study, faecal matter of *R. rattus* and *Mus musculus* infected with *H. nana* identified from different structures viz grocery shops, sweet shops, rice godowns, wheat godowns and poultry feed stores ranged from 2.97 (poultry feed stores) to 3.89% (grocery shops). Abu-Madi *et al.* (2001) reported the prevalence of *H. diminuta* in 17.1% rats in Doha, Qatar. The parasites of wild rats on a farm in UK included 22% *H. diminuta* eggs and 11% *H. nana.* The difference among the prevalence of *H. nana* in different studies may be due to selection of different rat species.

In the present study, it was noted that the faecal matter of the structures viz. grocery shop, sweet shops, rice godowns and wheat godowns of Sargodha district were significantly more infested with *E. coli, S. typhi* and *H. nana* compared to the same structures present in Faisalabad district. This might be assumed that the hygienic condition of the surroundings or vicinity of the structures of Sargodha from where samples were collected were poor compared to the vicinity of the structures of Faisalabad district.

Based on the results of the present study it can be concluded that the *R. rattus* and *Mus musculus* may pose a potential risk in the zoonosis of colibacilosis, salmonelosis and infestation of *H. nana*.

REFERENCES

Abu-Madi, M. A., J. W. Lewis, M. Mikhail, M. E. El-Nagger and J. M. Behnke, 2001. Monospecific helminths and arthropod infections in an urban population of brown rats from Doha, Qatar. J. Helminthol., 75: 313-320.

Athar, M., G. Muhammad, A. Shakhoor, M. Saqib, R. Ahmad and A. Naureen, 2007. A preliminary study on the effect of inactivated polyvalent mastitis vaccines on milk quantity and quality in dairy buffaloes. Pakistan Vet. J., 27(2): 85-91.

Battersby, S. A., R. Parsons and J. P. Webster, 2002. Urban rat infestations and the risk to public health. J. Environ. Hlth. Res., 1: 4-12.

Cappucino, J. G. and N. Sherman, 1996. Microbiology: A Laboratory Manual. The Benjamin/Cummings Publishing Co. Inc., USA, pp: 137-149.

Cizek, A., P. Alexa, I. Literak, J. Hamnk, P. Novak and J. Smola, 1999. Shiga toxin producing *Escherichia coli* in feedlot cattle and Norwegian rats from a

large scale farm. Letters App. Microbiol., 28: 435-440.

Cruickshank, R., J. R. Marman and R. H. A. Swain, 1975. Medical Microbiology. 12th Ed., Vol-II, Churchill Livingstone, Edinburgh, UK.

Hayat, S. and M. Akhtar, 1999. Parasitic Diagnosis. 1st Ed., University Grants Commission, Islamabad, Pakistan, pp: 15-27; 64-72.

Healing, T. D., 1991. Salmonella in rodents: A risk to man. J. Centre Dev. Res., 131: 114-116.

Henzler, D. J. and H. M. Optiz, 1992. The role of mice in the epizootiology of *Salmonella enteritidis* infection on chicken layer farms. Avian Dis., 36: 625-631.

Hilton, A. C., R. J. Willis and S. J. Hickie, 2002. Isolation of salmonella from urban wild brown rats (*Rattus norvagicus*) in the west Midlands, UK. Intl. J. Environ. Hlth. Res., 12: 163-168.

Linardi, P. M. and J. R. Botelho, 2002. Prevalence of *Trypanosoma lewisi* in *Rattus norvagicus* from Belo Horizonte, State of Minas Gerais, Brazil. Proc. Mem. Inst. Oswaldo. Cruz, Rio de Janeiro., 97: 411-414.

Ruiz, A., 2004. Rodents in disasters. Article for Vaccination Program. Pan American Health Org., USA, pp: 18.

Singleton, R. G., L. Smythe, G. Smith, D. M. Spratt, K. Aplin and A. L. Smith, 2003. Rodent diseases in South East Asia and Australia. Biol. Linn. Sol., 84: 565-583.

Wesbster, J. P., 1996. Wild brown rat (*Rattus norvagicus*) as a zoonotic risk on farms in England and Wales. Commun. Dis. Report, 6: 6-9.

Yaqoob, E., I. Hussain and S. U. Rahman, 2007. Molecular characterization by using random amplified polymorphic DNA (RAPD) analysis of *Salmonella enteritidis* isolates recovered from avian and human sources. Pakistan Vet. J., 27(2): 102-104.