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## RESEARCH ARTICLE

## DETECTION AND ISOLATION OF ZONOTIC PARASITES FROM AMERICAN COCKROACHES IN HOUSE HOLD OF KOHAT DIVISION, KHYBER PAKHTUNKHWA PAKISTAN

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### Abstract

The American Cockroaches transmit Zoonotic Parasites and other infectious agents to the human beings in their household in Khyber Pakhtunkhwa (KPK), Pakistan. The present study was carried out to detect these health hazards. Conventional Techniques were used to detect the prevalence of *Giardia*, *Cryptosporidium* and *E. histolytica* in Urban and Rural areas in Kohat, Khyber Pakhtunkhwa. A total of 300 samples were examined, among 40% (40/100) of Urban areas, 50% were positive for *Giardia*, 20% were positive for *Cryptosporidium* and 30% were positive for *E. histolytica*. Among 55% (110/200) of rural areas, 63.6% were positive for *Giardia*, 9.09% were positive for *Cryptosporidium* and 27.2% were positive for *E. histolytica*. The study revealed that American Cockroaches are the transmissible agent of the infectious agents which cause diseases /disturbance.

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### Introduction

Arthropods are probably the most successful of all animals because of one or other reasons. They are found in every type of habitat and in all regions of the world. They feed on a wide variety of plant or animal material and have been known as major cause diseases for Centuries and transmit the varieties of pathogens. Cockroaches, Blattodea order insects, act as an important mechanical vector for lots of pathogenic microorganism agents, including bacteria, protozoa, worms, fungi, and viruses amongst humans and animals.

In Bangladesh, *Salmonella*, *Shigella*, *S. aureus*, *B. cereus*, and *E. coli* were isolated from cockroaches (Paul, *et al.*, 1992).

*Cryptosporidium spp* and *Giardia duodenalis* are two protozoans parasites that affect human and wide range of domestic and wild animals (Adam, 2001). The *Cryptosporidium* genus comprises of more than 20 species, 12 of which are reported to infect mammals. The transmission of these two parasites is sustained by zoonotic (animal to human) and

anthropothonic (human to human) cycles. (Caccio and Rayan, 2008). Food and water is the major transmission route of *Cryptosporidium* and *Giardia* carried by Cockroaches, where they can resist and remain infective due to their robust form, the oocyst and cyst, respectively (Thompson, 2000; Fayer, *et al.*, 2000). The infected host shed in the environment a large number of these transmissible and infective stages, contributing to an increase of environmental contamination. Cyst and oocysts not only remain infective for long periods in environment but are also resistant to conventional treatment processes of water, representing a serious problem of Public health (Caccio, *et al.*, 2003; Lobbo, *et al.*, 2009). This problem is also potentiated by the fact that the number of parasites required to induce infection is small, i.e., infection dose of 82-123 oocysts for *Cryptosporidium spp* and 19-50 cysts for *Giardia spp* (Adam, 2001; Chappell, *et al.*, 2006).

The seasonal and environmental changes have great affect on incidence of the disease and extreme rainfall experienced around the world, as well as

globalization are some other factors affecting the transmission of Cryptosporidiosis and Giardiasis. Usually, food borne diseases go undetected and/or unreported, and there is a great need for improving and tracking of these pathogens in Cockroaches and also in other arthropods.

## METHODS

### Study Design

The study was carried out in Kohat Khyber Pakhtunkhwa for the detection of *Giardia*, *Cryptosporidium*, *Entamoeba Histolytica* parasites in cockroaches in the six localities of Project areas. The sites from where cockroaches were collected were Can't, KDA, Jarwanda, Jungle khail, Jarma and suliman talab. The samples were collected from 15th September, 2012 to 20th November, 2012.

### Collection and Identification

A total of 300 of cockroach samples were collected from six different localities for the detection of *Giardia*, *Cryptosporidium* and *E. histolytica*. The group of insects captured (mostly at night time or early morning) from the floor of wards and kitchens, basements or bathrooms of residential areas. Each cockroach was collected using a sterile test tube and sterile hand-gloves, transport to the laboratory and killed in a sterile jar using chloroform soaked cotton. Examined under the dissecting microscope and identified using standard taxonomic keys. The specie was identified as *Periplanata americana*.

### Media preparation

We prepare media of two types. One is Nutrient medium and the other is Macconkey's medium.

### Nutrient medium

Weight 28 gram of powder disperses in 1 litre of de-ionized water; allow soaking for 10 minutes. Then we wrap the nozzle of the flask with aluminium sheet. Then Swirl to mix then sterilize by autoclaving for 15 minutes at 121°C. Cool to 47°C. Mix well then pour plates.

### Macconkey's medium

Suspend 50g of medium in 1 litre of purified water. Heat with frequent agitation and boil for 1 minute to completely dissolve the medium. Then also we wrap the nozzle of this flask with aluminium sheet. Autoclave at 121°C for 15 minutes. Then take 100 glass petri dishes, tightly packed with paper in 5 sets, each containing 2 petri dishes. Autoclave all these at 121°C for 30 minutes. After autoclave we cool the medium and then pour in such away to minimize the air bubbles with great care. Out of hundred, 50

containing nutrient agar while remaining containing Macconkey's agar about 1-2mm thick layer. Then we place into the refrigerator.

### Culturing Media

All samples for bacteriology have been taken directly from external surfaces and feces pellets of insect *P.americana*. The procedure of Vazirianzadeh *et al.*, was followed to prepare the samples for culturing: The cockroach samples were washed by distilled water two times and for one minute in each time. The cockroach was transferred to a culture media Nutrient agar (Hi Media, India) and MacConkey's agar (Merck, Germany) plates under sterile conditions. Then we rub the cockroaches on these culture media, some in a zig-zag manner and other in a linear fashion. The various bacteria growth on the agar media were identified by colonial morphology on agar plates, Gram stains characteristics.

### Preparing Slides

For microscopy of zoonotic parasites associated with cockroaches, we prepare slides. First of all, we isolate the feces by pressing the abdomen of cockroach, and rub it gently on slides using tooth pick. Allow it to dry for few minutes, and then dip it in Ethanol for 5-10 minutes. After drying again dip it in Gimsa's stain for 5 minutes. Then washed it with distilled water. We prepare some slides of concentrated feces and some of dilute feces by adding small amount of water in it. We also prepare slides of colonies growing in agar medium. We took a small part of colony on slide and repeat the same procedure of preparing slides as mentioned above.

### Microscopy

For accurate vision, we dropped a small drop of emersion oil on slides. And took images of corresponding parasites from 10X to 100X and tally them with their original photographs available on Net.

## RESULTS

The American cockroaches (*Periplanata americana*) has transmit zoonotic Parasites and other infectious agents to the human beings in their house hold in Khyber Pakhtunkhwa. *Cryptosporidium spp* and *Giardia spp* are two protozoan parasites associated with these Cockroaches that effect humans and a wide range of domestic and wild animals. These parasites are the major cause of diarrheal disease in human and animal worldwide, causing high morbidity in their host; they can lead to death (Adam 2001).

The present study revealed that 300 samples of American cockroaches were examined, among these samples 100 were of urban areas and 200 were of rural areas. Overall prevalence of parasites was 50% (150/300) in which prevalence in Urban was 40% (40/100) and rural areas was 55% (110/200). Among 40% (40/100) of urban areas, 50% were positive for *Giardia*, 20% were positive for *Cryptosporidium* and

30% were positive for *E. histolytica*. Among 55% (110/200) of rural areas, 63.6% were positive for *Giardia*, 9.09% were positive for *Cryptosporidium* and 27.2% were positive for *E. histolytica*. The result showed that rural areas were more contaminated with parasitic diseases associated with Cockroach than the urban areas.

**Table 1: Prevalence rate of Zoonotic Parasites from American Cockroaches in House Hold of Kohat Division**

| S.No | Spp/Parasite           | Total Samples | Detection of Parasites | %age |
|------|------------------------|---------------|------------------------|------|
| 1.   | <i>Giardia</i>         | 300           | 70                     | 23.3 |
| 2.   | <i>Cryptosporidium</i> | 300           | 10                     | 3.3  |
| 3.   | <i>E. histolytica</i>  | 300           | 40                     | 13.3 |
| 4.   | Fungi                  | 300           | 30                     | 10   |

Grand Total of Detected Parasites: 150

**Table 2: Total Samples collected in Urban and Rural Areas of Kohat Division**

| S.No | Area  | Samples Collected | Detection of Parasites | %age |
|------|-------|-------------------|------------------------|------|
| 1.   | Urban | 100               | 40                     | 40   |
| 2.   | Rural | 200               | 110                    | 55   |

**Table 3: %age of Detective Parasites in Urban and Rural Areas**

| S.No | Area  | Spp/Parasites          | Detected Parasites | %age |
|------|-------|------------------------|--------------------|------|
| 1.   | Urban | <i>Giardia</i>         | 20                 | 50   |
|      |       | <i>Cryptosporidium</i> | 8                  | 20   |
|      |       | <i>E. histolytica</i>  | 12                 | 30   |
| 2.   | Rural | <i>Giardia</i>         | 50                 | 63.6 |
|      |       | <i>Cryptosporidium</i> | 10                 | 9.09 |
|      |       | <i>E. histolytica</i>  | 30                 | 27.2 |

## DISCUSSION

The American cockroaches (*Periplanata americana*) has transmit zoonotic Parasites and other infectious agents to the human beings in their house hold in Khyber Pakhtunkhwa. Many people considered them as less infectious but these are risk factors and health hazards to human beings. *Cryptosporidium* spp and *Giardia* spp are two protozoan parasites associated with these Cockroaches that effect humans and a wide range of domestic and wild animals. These parasites are the major cause of diarrheal disease in human and animal worldwide, causing high morbidity in their host; they can lead to death (Adam 2001).

The present study reported that *Cryptosporidium* spp and *Giardia* spp were widely distributed in Kohat. This was a novel work which was carried out for the first time in Kohat. A total of 300 samples of Cockroaches were examined, amongst these samples 100 were of urban areas and 200 were of rural areas.

Overall prevalence of parasites was 50 percent (150/300) in which prevalence in urban area was 40 percent (40/100) and rural area was 55 percent (110/200). Among 40 percent (40/100) of urban areas, 50 percent were positive for *Giardia*, 20 percent were positive for *Cryptosporidium* and 30 percent were positive for *E. histolytica*. Among 55 percent (110/200) of rural areas, 63.6 percent were positive for *Giardia*, 9.09 percent were positive for *Cryptosporidium* and 27.2 percent were positive for *E. histolytica*. Result of this study revealed that the prevalence of parasites was greater in rural area than in urban area.

In the current study some isolated bacteria from *P. aegyptica*, both external body and feces pellets, are pathogenic. These bacteria are for the first times which are reported from the external body and faeces pellet of *P. aegyptica*. They are *E. coli*, *K. pneumoniae*, *B. cereus*, *B. subtilis* and *S. aureus* of external body and same species of feces pellets. In

another study which was conducted by Tachbele *et al.*, 2006 in hospitals and restaurants of Ethiopia the isolated pathogenic bacteria were *Salmonella* species, *Shigella flexneri*, *E. coli*, *S. aureus* and *B. cereus*. The specimens were taken from digestive systems of *B. germanica*. There is a more similarity between the fauna of bacteria of faeces pellets in the present research. (Elgderi *et al.*, 2006) have isolated 25 species of potential pathogen from the hospital and household cockroaches, respectively, with *Klebsiella*, *Enterobacter*, *Serratia* and *Streptococcus* predominant from Libya. They have revealed that the place of collecting the cockroaches can affect the results according to transmitted bacteria. *Klebsiella* and *Enterobacter* were common among the household cockroaches than among the hospital. The result of (Elgderi *et al.*, 2006) is similar to the present study from the point of isolating of *Klebsiella* and *Enterobacter*.

In another study which was carried out in three hospitals of Iran by (Karimi Zarchi and Vatani, 2006), the most common species of bacteria isolated from cockroaches were *E. coli*, *Streptococcus* group D, *Bacillus* species, *K. pneumoniae*, and *Proteus vulgaris*. These species of bacteria were similar to the current study results. There is very less similarity between present study and the study of Vahabi *et al.*, 2007, which they are shared only in *E. coli* from external part of cockroaches.

The results of the present study revealed contamination of almost all cockroaches collected from homes with different parasites which are significantly higher in comparison to control group. The importance of cockroaches as carrier of parasitic worm, cysts, or eggs, is because there are some reports about the presence of parasitic forms on or in cockroaches (Greenberg, 1973). The finding of the present study showed the parasitic contamination in high numbers. The presence of *E. vermicularis* infestation indicates that the cockroaches had opportunity to get in touch with infested patients or contaminated clothes which emphasizes their vectorial potential for parasitic diseases (Chan *et al.*, 2004).

*Salmonella* has been isolated from different species of cockroaches found in hospitals, restaurants, residents, schools, animal shelters etc. throughout the world (Rivault *et al.*, 1993, Fathpouer *et al.*, 2003). The fact that 10 of the 12 *Salmonella* isolates were from the gut suggested that cockroach intestine served as a major reservoir of *Salmonella*. Moreover, 11 of the isolates were from hospital cockroaches and were found to be resistant to 3 or more drugs, suggesting the possible role of cockroaches as reservoir and vectors of drug resistant *Salmonella* in health facilities that may contribute to nosocomial

infections. Isolation of drug resistant salmonellae and other pathogens from hospital cockroaches has been reported by various workers elsewhere (Fotedar *et al.*, 1991, Rivault *et al.*, 1993). Although food handlers are claimed to play the major role in the transmission of *Shigella*, different authors have reported the presence of *Shigella* spp. in cockroaches found in hospitals, restaurants and residences indicating their importance in the dissemination of the bacterium (Oothuman 1989, Paul 1992, Agbodaze and Osuwusu 1989). Our *Shigella flexneri* isolates, obtained from restaurant cockroaches, were resistant to 7 antimicrobials. This indicated that *B. germanica* could be a potential vector in spreading multi-drug resistant *Shigella* in food establishment areas in Ethiopia. Our isolation of *S. aureus* from *B. germanica* collected from hospitals and restaurants is in agreement with other findings elsewhere (Paul *et al.*, 1992, Oothuman *et al.*, 1989, Burgess 1984, Le Guyader *et al.*, 1989, Prado *et al.*, 2002). The isolation of almost proportional numbers of *S. aureus* from hospital and restaurant cockroaches is indicative of the potential role of cockroaches in the dissemination of *S. aureus* in hospitals and food catering centers alike. Furthermore, all *S. aureus* isolates were resistant to all antibiotics tested except gentamycin suggesting the wide-spread distribution of multiple drug resistant *S. aureus* in the environment and the potential role of cockroaches in spreading such strains. In this study, although the number of isolates tested was small, an increase in resistance was seen when compared to previous studies in Tikur Anbessa Hospital and elsewhere in Ethiopia (Gedebou 1982, Gedebou *et al.*, 1987). Fathpour *et al.*, 2003). We could not demonstrate whether our *B. cereus* isolates from cockroach intestine or external parts were vegetative forms or spores. Their introduction onto appropriate type of food by cockroaches would, however, result in their proliferation and production of enterotoxins. The observation of various resistance patterns among our *B. cereus* isolates agreed with that in another study (Drobniewski, 1993). The commonest pattern was, however, resistance to four particular drugs. Since *B. cereus* produces  $\beta$ -lactamase, it is expected to be resistant to penicillin and oxacillin. *B. cereus* is more of a toxin producer than infectious. Thus, its observed resistance to various drugs may not be of great concern. Although the mechanical transmission of pathogens has received considerable attention among researchers, few attempts have been made to determine whether cockroaches sustain internal infections after in an extended period of time. Various workers have shown that cockroaches could maintain and excrete viable pathogens for many days. In our study, cockroaches fed with *Salmonella* could shed the pathogen for 35

days after which all the test cockroaches were dead. It is evident from this result that *B. germanica* is capable of ingesting *Salmonella* contaminated food and excreting viable bacteria in its faeces.

When compared with other results, this is probably the longest *Salmonella* excretion time, and we assume that the cockroaches could have continued to excrete the pathogen for an extended period of time if they had lived longer. Since our *Salmonella* test strains were recovered only after enrichment, the number of cells shed by the infected cockroaches must have been very low. Our *S. aureus* test strain could also survive in cockroach gut for two weeks. However, the increasing difficulty to isolate *Salmonella* and *S. aureus* from the faecal pellets with time indicated that the pathogens were not multiplying in the gut of cockroach. The observed survival and shedding rate of our test strains in cockroach gut may not hold true for other strains because the phenomena seem to be associated with bacterial strain, species of cockroaches and antagonism effects of endogenous gut bacteria as observed in other studies (Klowden and Greenberg 1976). This may also explain the inability of our *Shigella* test strain to survive in cockroach gut even for a day.

## CONCLUSION

It can be concluded that Cockroaches transmit several pathogens to human being. Cockroaches also causing health hazards asthma like diseases in the women of rural community.

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