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Occurrence of *Escherichia coli* in Wildlife from Different Habitats of Sarawak, Malaysia

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ABSTRACT

This study was carried out to assess the occurrence of *Escherichia coli* (*E. coli*) in the bats, birds and rodents as representative of wildlife from different habitats in Sibu and Kapit, Sarawak, Malaysia. A total of 682 swab samples were collected from wildlife hosts and screened for the bacteria *E. coli* and *E. coli* O157:H7 using standard microbiological methods and molecular techniques. The overall occurrence rates of *E. coli* among these hosts were 14%, 17% and 54% for bats, birds and rodents, respectively. The occurrence of *E. coli* was the highest in rodents regardless of the habitats. Isolated *E. coli* were then screened for *E. coli* O157:H7 by using a multiplex PCR with four primer pairs targeting for Shiga toxin producing genes (*slt*-I and *slt*-II), and the genes involved in biosynthesis of O157 antigen (*rfb*E) and H7 antigen (fliC$_{H7}$). *slt*-I, *slt*-II and *rfb*E genes were not detected in any of the *E. coli* isolates. However, the gene encoding for H7 antigen was detected in 23 *E. coli* isolates. This indicated that *E. coli* O157:H7 strain was not detected in the wildlife studied. Absence of *E. coli* O157:H7 in the wildlife studied indicated these wild animals do not serve as an important reservoir of *E. coli* O157:H7. However, precautions have to be taken as other group of pathogenic *E. coli* may pose a zoonotic risk for humans and other animals.

Keywords: *Escherichia coli*, occurrence, Sarawak, wildlife

INTRODUCTION

Escherichia coli belong to the family *Enterobacteriaceae* and is part of the normal microflora of the gastrointestinal tract of mammals and birds. However, some strains are identified as pathogenic *E. coli* by their ability to possess specific virulence factors and specific toxin-encoding genes (Nataro & Kaper, 1998). Virulence factors for *E. coli* O157:H7 include the production of one or more shiga-toxin (Mead & Griffin, 1998). Enterohemorrhagic *E. coli* O157:H7 are zoonotic pathogens associated with severe human illness such as hemorrhagic colitis, hemolytic uremic syndrome and thrombocytopenic thrombotic purpura (Caprioli *et al.*, 2005).

E. coli O157:H7 was first recognized as a pathogen in 1982 during an outbreak of hemorrhagic colitis caused by the consumption of undercooked meat in Oregon and Michigan (Riley *et al.*, 1983). Ground beef, cattle and other bovine sources have been identified as the main natural reservoir for *E. coli* O157:H7. Birds and rodents have also been reported to harbour *E. coli* O157:H7. For example, Wallace *et al.* (1997) reported the isolation of *E. coli* O157 from wild birds in Morecambe Bay and Lancaster, UK which implicated that wild bird can serve as potential vectors for the dissemination of *E. coli* O157:H7. Other animals such as swine in the United State also harboured potentially pathogenic *E. coli* O157 (Feder *et al.*, 2003).

Polymerase Chain Reaction (PCR) assay represents good alternative to traditional typing methods for the diagnosis of Shiga toxin producing *E. coli* due to their simplicity,

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rapidity and specificity (Olsvik & Strockbine, 1993). Multiplex PCR allows simultaneous detection of two or more different virulence genes in a single reaction, inevitably leads to a greater number of positive samples compared to conventional methods (Gallian, 2003). Multiplex PCR have been successfully employed to detect *E. coli* O157:H7 (Apun *et al.*, 2003; Hu *et al.*, 1999; Radu *et al.*, 2001).

The surveillance of the epidemiology of these pathogenic microorganisms in wildlife is particularly important due to the increasing activities of translocation of wild animals which increased the possibility of disseminating the pathogenic bacteria. In addition, not much is known about the distribution of *E. coli* in wildlife of different ecological niche in Sarawak. Although we have reported the occurrence of *E. coli* in the selected wildlife from disturbed habitats in Sibu, Sarawak (Apun *et al.*, 2011), to the best of our knowledge, there is no report on undisturbed forest in Sarawak. The surveillance of pathogenic enterobacteria in different ecological niche is important as the development has been reported to affect the diversity and distribution of microorganisms in the animal hosts (Gordon & Fitzgibbon, 1999). Therefore, this study was conducted to survey the occurrence of *E. coli* in birds, bats and rodents from the five different sampling areas located in Sarawak. Birds, bats and rodents were chosen to represent wildlife due to previous reports that these animals can act as reservoirs for *E. coli* O157:H7 (Foster *et al.*, 2006; Nielsen *et al.*, 2004; Wallace *et al.*, 1997).

METHODOLOGY

Sample collection

Small mammals and bird specimens were collected from five sampling habitats located in Sibu and Nanga Merit, Kapit during June 2008 and June 2009, respectively. Specimens were collected from a forest park which is a recreational park and an oil palm plantation area, in Sibu; as well as a human settlement area and a forest area located in Nanga Merit, Kapit, Sarawak. Both of the forest and recreational parks in Sibu consisted of urban forests located 10 km from Sibu Town. The oil palm plantation in Sibu is a peat swamp area surrounded by oil palm and rubber tree plantations. Nanga Merit is about 70 km from Kapit. The human settlement area, Nanga Merit is located along the bank of Rajang River, whereas the forest area with minor human habitation and activities is located near the bank of Beletik River, upstream of Merit River. Avian and fruit bats were captured using standard mist nets whereas insect bats were captured using harp traps. Cage traps were used to capture rodents. Identification of mammals and birds was done according to Payne *et al.* (1985) and Francis (2005), respectively**.**

Anal swabs of small mammals and cloacal swabs from avian specimens were collected using wet sterilised cotton buds and immediately stored in 900 µl phosphate buffered saline (PBS). All the samples were kept at 4°C until processed, usually within the same day. All of the samples were cultured directly on MacConkey agar (Oxoid, England) and incubated aerobically at 37°C for 24 hours at the sampling site. Isolates tentatively identified as *E. coli* were further plated on EMB agar (Oxoid, England) after taken back to the laboratory. Plates were examined for the presence of colonies with metallic green sheen, and colonies that exhibited this characteristic were stored in nutrient agar (Oxoid, England).

Bacteria Identification

All isolates were tested using gram-staining and a series of standard biochemical tests. The biochemical tests included Kligler Iron Agar, IMVIC test and motility test. *E. coli* reference cultures ATCC 25922 was included as a positive control. The bacteria were identified and the results were compared to the Bergey's Manual of Systematic Bacteriology (Krieg *et al.*, 1984). The bacteria were further confirmed by using commercial identification kits, API 20E system (BioMerieux, France).

DNA Isolation

Bacterial total DNA of *E. coli* isolates was extracted using boiling extraction method as described by Gallian (2003) with minor modification. For the extraction of bacterial genomic DNA, *E. coli* isolates was cultured in 5 ml Luria-Bertani broth (BBL, United States) overnight at 37^{o}C with agitation at 300 *g*. Overnight broth suspensions (1 ml) were then

centrifuged at 19,000 *g* for 5 minutes to collect the pellets. Pellets were re-suspended in 100 µl of distilled water and boiled for 20 minutes. The lysed cells were then cooled on ice for another 20 minutes and centrifuged at 19,000 *g* for 3 minutes. The supernatant containing DNA was used as template in the multiplex PCR assay.

Multiplex PCR assay for the detection of *E. coli* O157:H7

Multiplex PCR assay for genes encoding Shiga toxin I and II, antigen O157 and antigen H7 were performed in a single tube reaction. Four set of primers synthesized commercially by FIRST BASE Laboratory Sdn. Bhd. were used in this study. SLT-I and SLT-II primer sets are targeted for the Shiga toxin producing genes (*slt*-I and *slt*-II) while Rfb and $FLIC_{h7}$ primer sets are targeted for the genes involved in biosynthesis of O157 antigen and H7 antigen, respectively. *E. coli* O157:H7 reference strains EDL 933 was included as a positive control. The primers used for the amplification of the Shiga-toxin genes were described by Meng *et al.* (1997) and the detection of *rfb*E gene and $fliC_{H7}$ gene were as previously reported by Gannon *et al.* (1997).

The condition for the multiplex PCR assay performed was as described by Hu *et al.* (1999). Each PCR was performed in a total reaction volume of 25 µl containing 1 X PCR buffer, 2.5 mM Magnesium Chloride ($MgCl_2$) (Promega Corp, USA), 0.2 mM dNTP mix (Promega Corp, USA), 0.2 µM of primers SltI-F/SltI-R, SltII-F/SltII-R, Rfb-F/Rfb-R, $FLIC_{H7}$-F/$FLIC_{H7}$-R, 1 Unit Go*Taq*@ Flexi DNA polymerase (Promega Corp, USA) and 10 µl of DNA template. PCR amplification was performed using iCycler™ Thermal Cycler (Bio-Rad, USA) as follows: Initial denaturation at 94°C for 5 minutes; 35 cycles of denaturation at 94°C for 30 seconds; annealing at 59°C for 1 minute; and extension at 72°C for 1 minute; followed by final extension at 72°C for 7 minutes.

Gel electrophoresis

A 10 µL aliquot of each amplification product was analyzed using electrophoresis on 2% agarose gels cast and run in 1 X TAE buffer. A 100-bp marker (Vivantis, Malaysia) was included in the gel. Gel was stained with ethidium bromide (0.5 µg/ml) and visualized using transmitted ultraviolet illumination and photographed using gel documentation system (AlphaDigiDoc RT).

DNA sequencing

The *E. coli* isolates that produced $fliC_{H7}$ amplicon were sent for sequencing to determine the strain identity of the *E. coli* isolates. The amplicon of positive control *E. coli* O157:H7 reference strain EDL 933 was also sequenced as a control. PCR mixture was prepared in 75 µl reaction volume. After PCR amplification, the amplified 625 bp product was purified from 2.0% agarose gels using a Gel Extraction Kit (Fermentas), according to the manufacturer's instructions. The purified fragment was verified via agarose gel electrophoresis. The confirmed purified products were sent to a commercial DNA sequencing service company (First Base, Malaysia).

Analysis of nucleotide sequences for homology search was performed using BLASTN 2.2.23 (Basic Local Alignment Search Tool) GenBank database available from National Center for Biotechnology Information (NCBI).

RESULTS

In this study, swab samples were collected from a total of 105 birds, 84 bats and 44 rodents in Sibu and 208 birds, 224 bats and 17 rodents in Nanga Merit and tested for the occurrence of *E. coli*. A total of 106 and 259 isolates of *E. coli* were isolated from wildlife collected from Sibu and Kapit respectively. The occurrences of the *E. coli* in wildlife from Sibu and Nanga Merit, Kapit are illustrated in Table 1 and 2.

The overall occurrences of *E. coli* isolated from the three sampling sites in Sibu were recently reported by Apun *et al.* (2011). In Nanga Merit, the overall occurrences of *E. coli* isolated from both sampling sites were 15%, 17% and 82% for bats, birds and rodents, respectively.

A total of 47 and 82 *E. coli* isolates from Sibu and Nanga Merit respectively were tested in PCR assay. Of 129 isolates tested, none of the isolates generated *stx*1, *stx*2, and *rfb*E gene

with the expected band size of 210 bp, 292 bp and 484 bp respectively. $Flic_{H7}$ gene of expected size of 625 bp was detected in 14 *E. coli* isolates from Sibu and nine *E. coli* isolates from Nanga Merit as shown in Figure 1 and Figure 2. These isolates were recovered from three bats hosts, three bird hosts, eight rodent hosts in Sibu and five bats hosts, and four rodent hosts in human settlement area in Nanga Merit. Hence, the absence of *stx*1, *stx*2, and *rfb*E genes indicated that none of the *E. coli* isolates were *E. coli* O157:H7.

Table 1. Occurrence of *E. coli* in wildlife from Sibu, Sarawak.

Sampling site (Sibu)	Animal Source					
	Birds (n = 105)		Bats (n = 84)		Rodents (n = 44)	
	No of samples tested	No (%) positive for *E. coli*	No of samples	No (%) positive for *E. coli*	No of samples	No (%) positive for *E. coli*
Forest Park	30	8 (27)	17	0	11	4 (36)
Recreational Park	19	6 (32)	39	8 (21)	10	4 (40)
Oil palm Plantation	56	5 (9)	28	1 (4)	23	11 (48)

Table 2. Occurrence of *E. coli* in wildlife from Nanga Merit, Kapit, Sarawak.

Sampling site (Nanga Merit)	Animal Source					
	Birds (n = 208)		Bats (n = 224)		Rodents (n = 17)	
	No of samples tested	No (%) positive for *E. coli*	No of samples	No (%) positive for *E. coli*	No of samples	No (%) positive for *E. coli*
Human Settlement	102	28 (28)	93	28 (30)	17	14 (82)
Recreational Park	106	7 (7)	131	5 (4)	0	0

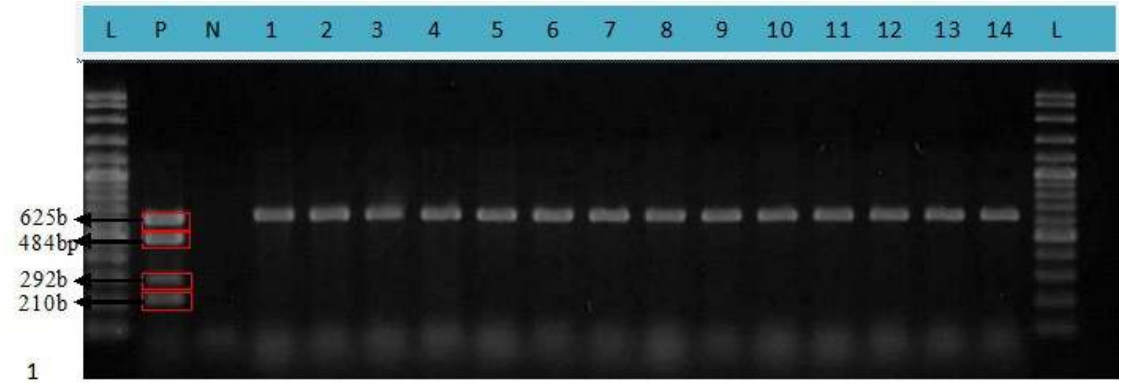

Figure 1. Amplicon obtained by multiplex PCR for the 14 *E. coli* isolates isolated from Sibu with $fliC_{H7}$ gene with expected size of 625 bp fragmented by 2% agarose gel electrophoresis. **Lane L**: 100 bp ladder, **Lane P**: *E. coli* O157:H7 reference strains EDL933 as positive control, **Lane N**: Negative control, **Lane 1**: Isolate (C0813) from bat sample in recreation park, **Lane 2**: Isolate (TK153581) from bat sample in recreation park. **Lane 3**: Isolate (TK153583) from bat sample in recreation park, **Lane 4**: Isolate (BA025) from bird sample in recreation park, **Lane 5**: Isolate (A2110) from bird sample in forest park, **Lane 6**: Isolate (TK156080) from rodent sample in oil palm plantation, **Lane 7**: Isolate (PL007) from rodent sample in oil palm plantation, **Lane 8** : Isolate (PL010) from rodent sample in oil palm plantation, **Lane 9**: Isolate (PL011) from rodent sample in oil palm plantation, **Lane 10**: Isolate (PL0031) from rodent sample in oil palm plantation, **Lane 11**: Isolate (PL033) from rodent sample in oil palm plantation, **Lane 12**: Isolate (PL0035) from rodent sample in oil palm plantation, **Lane 13**: Isolate (PL0036) from rodent sample in oil palm plantation and **Lane 14**: Isolate (D0113) from bird sample in oil palm plantation.

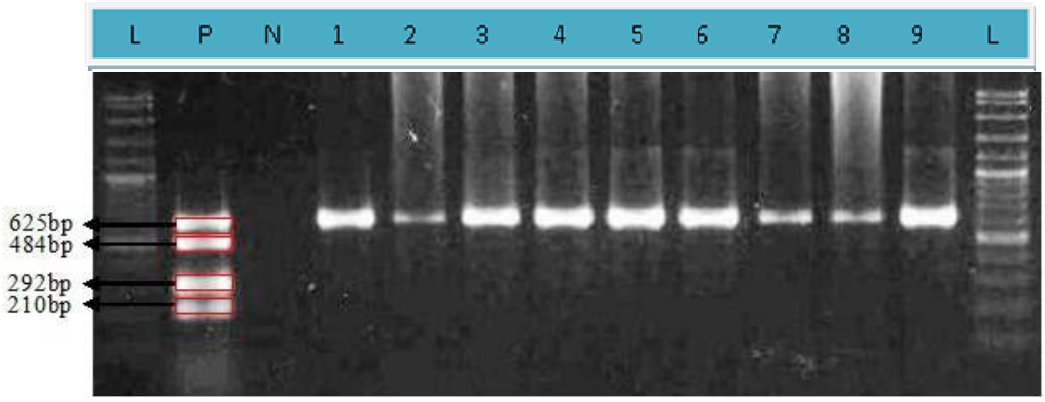

Figure 2. Representative amplicon obtained by multiplex PCR for the 9 *E. coli* isolates from Nanga Merit with $fliC_{H7}$ gene with expected size of 625 bp fragmented by 2% agarose gel electrophoresis. **Lane L**: 100 bp ladder, **Lane P**: *E. coli* O157:H7 reference strains EDL933 as positive control, **Lane N**: Negative control, **Lane 1**: Isolate (U2021) from rodent sample in human settlement, **Lane 2**: Isolate (U2023) from bat sample in human settlement, **Lane 3**: Isolate (U2032) from bat sample in human settlement, **Lane 4**: Isolate (U2037) from bat sample in human settlement, **Lane 5**: Isolate (U2044) from rodent sample in human settlement, **Lane 6**: Isolate (U2049) from rodent sample in human settlement, **Lane 7**: Isolate (U2081) from bat sample in human settlement, **Lane 8**: Isolate (U2082) from bat sample in human settlement, and **Lane 9**: Isolate (U2117) from rodent sample in human settlement.

Seventeen representatives of $fliC_{H7}$ amplicon-positive *E. coli* isolates were sent for sequencing for strain confirmation. Good sequences were obtained for all the $fliC_{H7}$ gene sequence with high nucleotide sequence homology of 98% to 100%. The amplicon of the positive control, *E. coli* O157:H7 strain EDL 933, matched with the $fliC_{H7}$ gene for EDL in Genbank entries. The $fliC_{H7}$ amplicon for *E. coli* isolated from the wildlife in recreational park, Sibu aligned with *E. coli* reference strains *E. coli* S88, *E. coli* UT189, *E. coli* APEC 01, *E. coli* U5-41 flagellin genes from GenBank. Other $fliC_{H7}$ amplicon for *E. coli* isolated from the wildlife in forest park, oil palm plantation and Nanga Merit human settlement area were aligned to *E. coli* reference strain 14097 flagellin gene from GeneBank.

DISCUSSION

In the five sampling habitats, rodents showed the highest occurrence of *E. coli* except for Nanga Merit forest area, where no rodent was captured. The occurrence of *E. coli* was always higher in rodents compared to birds and bats regardless of the habitats. The occurrence of *E. coli* in rodents (43%) captured in Sibu was generally in accordance with the findings previously reported by Gordon and Cowling (2001) where 42% of *E. coli* was detected in rodent hosts from Australia. Similarly, the occurrence of *E. coli* in birds captured in Sibu (18%) and Kapit (17%) was in general agreement with a study previously done by Gordon and Cowling (2001) in Australia where 23% of *E. coli* were found in the birds. The occurrences of *E. coli* in bats were 11% and 15% in Sibu and Nanga Merit, respectively. Adesiyun *et al.* (2009) reported a comparatively similar occurrence of *E. coli* (13%) in bats in Trinidad and Tobago.

Among all of the sampling habitats, the occurrence of *E. coli* in rodents was the highest in Nanga Merit human settlement. In human settlement area, rodents had the closest association with human and the contaminated environment. From our observations during the fieldtrip, in human settlement site there was no designated area for waste disposal and the rubbish were scattered surrounding the long houses. Every household in the village lacked a proper sanitary system. As rodents live in close proximity to human, their food source may come from human wastes, garbage, food waste, or sewage effluent. In addition, rodents live on land where they frequently come into direct contact with soil and fecal matter which also served as reservoir for enteric pathogen (Santamaria & Toranzos, 2003).

Subsequently, these factors could potentially lead to a higher possibility of rodents to acquire *E. coli* from the contaminated surroundings. Thus, the occurrence of *E. coli* was always higher in rodents.

The occurrence of the *E. coli* in birds was the second highest in all of the sampling habitats except for human settlement area in Nanga Merit where occurrence of *E. coli* in birds and bats were comparatively similar. The presence of *E. coli* in birds can be attributed to its feeding habits where the bacteria could be picked from rubbish tips, sewage outfalls and consumption of contaminated food as reported by a previous study (Wallace *et al.*, 1997). The occurrence of *E. coli* was high in the disturbed habitats with frequent human activities which consequently provide more opportunity for the birds to feed on contaminated food and water. According to Gordon and FitzGibbon (1999), animals such as birds living in close association with humans were more likely to have *E. coli* than animals living away from human habitation. Among all of the sampling sites, the occurrence of birds was the lowest in the forest area in Nanga Merit (7%) which has the least perturbation where there were no human habitation and minimal human activity.

The occurrence of *E. coli* in bats captured from the human settlement area in Nanga Merit was the highest. Majority of the bats harboured *E. coli* were insect bats. The feeding method of insect bats which were insectivorous might be one of the transmission routes for *E. coli*. Transmission of *E. coli* might also occur after ingestion of contaminated food or water. Investigations concerning the interaction between bacteria and insect showed that insects served as reservoir for pathogenic *E. coli* (Ahmad *et al.*, 2007; Khalil *et al.*, 1994). Thus, insects or contaminated water were possible sources for *E. coli*. Great population densities of bats and their crowded roosting behaviour increased the chances of intra-species or interspecies transmission of the bacteria.

In the PCR screening of the anal and cloacal samples of wildlife in Sarawak, none of the *E. coli* isolates was positive for *E. coli* O157:H7 strains as none of the isolates displayed genes encoding for *Shiga toxin 1*, *Shiga toxin 2* and gene involved in biosynthesis of O157 antigen. In a recent research done by Kobayashi *et al.* (2009), *E. coli* O157 was not detected from the *E. coli* isolates from wild birds in Tokyo Bay, Japan. Similarly, in the study of wild birds in Scotland, only one out of 231 samples was positive for *E. coli* O157:H7 (Foster *et al.*, 2006) which indicated a very low prevalence of STEC O157 in wild birds. *E. coli* O157:H7 was also absent from wildlife in Trinidad and Tabago (Adesiyun, 1999).

In the PCR screening, H7 flagellar gene was detected in 23 *E. coli* isolates. Flagella and motility are critical elements in the virulence strategies of many bacterial pathogens. Erdem *et al.* (2007) demonstrated that the H7 flagella possess adhesive properties, particularly the ability to bind mucins, which contributed to colonization of mucosal surfaces. The $fliC_{H7}$ amplicon for *E. coli* isolated from the wildlife in recreation park, Sibu aligned with those of *E. coli* reference strains *E. coli* IHE3034, *E. coli* S88, *E. coli* UT189, *E. coli* APEC 01, *E. coli* U5-41 flagellin genes from GenBank. These reference *E. coli* strains from GenBank were categorized as the extraintestinal pathogenic *E. coli*. Sequences analysis of the $fliC_{H7}$ gene of the *E. coli* isolates from wildlife (three bat hosts and a bird host) in recreation park, Sibu were suspected to be associated with extraintestinal pathogenic *E. coli* as they shared 99% homology in nucleotide sequences of the flagellin (fliC) gene from Genbank. The presence of $fliC_{H7}$ gene in the suspected extraintestinal pathogenic *E. coli* isolated from these four wildlife hosts suggested a possible cross-interaction among the bats and birds allowing the transmission of the *E. coli*. Bats and birds might be sharing the same food sources contaminated with the *E. coli* strains. There was a possibility that bats and birds came into direct or indirect contact in the same habitat and played an important role in the dissemination of the *E. coli* strain. Other $fliC_{H7}$ amplicon for *E. coli* isolated from the wildlife in forest park, oil palm plantation and Nanga Merit human settlement site were aligned to *E. coli* reference strain 14097 flagellin gene from GenBank. *E. coli* reference strains belongs to serotype *E. coli* O153:H7:K- (Machado *et al.*, 2000). *E. coli* O153:H7 was classified as enteropathogenic *E. coli* (Blanco *et al.*, 2006)

or enterohemorrhagic *E. coli* which had been reported to be isolated in rabbit (Garcia & Fox, 2003). Thus, the detection of these strains indicated a possible risk of *E. coli* being transmitted from the wildlife to the environment and subsequently creating a zoonotic risk for human.

CONCLUSION

In conclusion, *E. coli* was isolated among the wildlife hosts in all of the habitats studied. Among the animal hosts, the occurrence of *E. coli* was shown to be always higher in rodents compared to other wildlife studied regardless of the habitats. The absence of *E. coli* O157:H7 indicated that bats, birds, or rodents from these habitats are not important reservoirs in the epidemiologic cycle of emerging enteric bacterial zoonoses in the state of Sarawak, Malaysia. However, the findings of other pathogenic *E. coli* strains raise concerns about the zoonotic risks for humans, given that the wildlife are in close proximity with humans.

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REFERENCES

Adesiyun, A.A. (1999). Absence of *Escherichia coli* O157 in a survey of wildlife from Trinidad and Tobago. *Journal of Wildlife Disease*, 35:115-120.

Adesiyun, A.A., Johnson, A.S., & Thompson, N.N. (2009). Isolation of enteric pathogens from bats in Trinidad. *Journal of Wildlife Disease*, 45:952-961.

Ahmad, A., Nagaraja, T.G., and Zurek, L. (2007). Transmission of *Escherichia coli* O157:H7 to cattle by house flies. *Preventive Veterinary Medicine*, 80:74-81.

Apun, K., Chang, P.P., Sim, E.U.H., & Salleh, M.A. (2003). Survey of raw beef marketed in East Malaysia for *Escherichia coli* O157 and O157:H7. *Journal of Bioscience*, 14:49-56.

Apun, K., Kho, K.L., Chong, Y.L., Hashim, H. F., Abdullah, M.T., Rahman, M.A., Bilung, L.M., & Lihan, S. (2011). Detection of *Escherichia coli* O157:H7 in wildlife from disturbed habitats in Sarawak. *Research Journal of Microbiology,* 6:132-139.

Blanco, M., Blanco, J.E., Dahbi, G., Alonso, M.P., Mora, A., Coira, M.A., Madrid., C., Juarez, A., Bernardez. M.I., Gonzalez, E. A., & Blanco, J. (2006). Identification of two new intimin types in atypical enteropathogenic *Escherichia coli*. *International Microbiology*, 9:103-110.

Caprioli, A., Morabito, S., Brugere, H., & Oswald, E. (2005). Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Veterinary Research*, 36:289-311.

Erdem, A. L., Avelino, F., Xicohtencatl-Cortes, J., & Giro´ n, J.A. (2007). Host protein binding and adhesive properties of H6 and H7 flagella of attaching and effacing *Escherichia coli*. *Journal of Bacteriology*, 189:7426–7435.

Francis, C.M. (2005). *A pocket guide to the birds of Borneo*. Kota Kinabalu: Sabah Society.

Feder, I., Wallace, F.M., Gray, J.T., Fratamico, P., Paula, J., Fedorka-Cray, Pearce, R.A., Call, J.E., Perrine, R., & Luchansky, J.B. (2003). Isolation of *Escherichia coli* O157 from intact colon fecal samples of swine. *Emerging Infectious Disease*, 9:380-383.

Foster, G., Evans, J., Knight, H.I., Smith, A.W., Gunn, G.J., Allison, L.J., Synge, B.A., & Pennycott, T.W. (2006). Analysis of feces samples collected from a wild-Bird

garden feeding station in Scotland for the presence of verocytotoxin-producing *Escherichia coli* O157. *Applied and Environmental Microbiology*, 72:2265-2267.

Gannon, V.P.J., D'Souza S., Graham, T., King, R. K., Rahn, K., & Read, S. (1997). Use of the flagellar H7 gene as target in multiplex PCR assays and improved specificity in identification of enterohaemorrhagic *Escherichia coli* strains. *Journal of Clinical Microbiology*, 35:656-62.

Gallian, P. (2003). Detection and subtyping of shiga toxin producing *Escherichia coli* (STEC). In Sachse, K. and Frey, J. (Eds.). *PCR detection of microbial pathogens: Methods and protocols*. Totowa: Human Press Inc. Pp.163-183.

Garcia, A. & Fox. J.G. (2003). The rabbits as a new reservoir host of enterohemorrhagic *E. coli*. *Emerging Infectious Disease*, 9:1592-1597.

Gordon, D.M. & FitzGibbon, F. (1999). The distribution of enteric bacteria from Australian mammals: host and geographical effects. *Microbiology*, 145:2663-2671.

Gordon, D.M., & Cowling, A. (2001). The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. *Microbiology*, 149:3575-3586.

Hu, Y., Zhang, Q., & Meitzler, J.C. (1999). Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by a multiplex PCR. *Journal of Applied Microbiology*, 87:867-876.

Khalil, K., Lindblom, G-B., Mazhar, K., & Kaijser, B. (1994). Flies and water as reservoirs for bacterial enteropathogens in urban and rural areas in and around Lahore, Pakistan. *Epidemiology and Infection*, 113:435-444.

Kobayashi, H., Kanazaki, M., Hata, E., & Kubo, M. (2009). Prevalence and characteristics of eae- and stx- positive strains of *Escherichia coli* from wild birds in the immediate environment of Tokyo Bay. *Applied and Environmental Microbiology*, 75:292-295.

Krieg, N.R., Holt, J.G., Murray, R.E.G., Brenner, D.J., Bryant, M.P., Moulder, J.W., Pfennig, N., Sneath, P.H.A., & Staley, J.T. (1984). *Bergey's Manual of Systematic Bacteriology Volume 1*. United States: A Waverly Company.

Machado, J., Grimont, F., & Grimont, P.A.D. (2000). Identification of *Escherichia coli* flagellar types by restriction of the amplified fli*C* gene. *Research in Microbiology*, 151:535-546.

Mead, P.S. & Griffin, P.M. (1998). *Escherichia coli* O157:H7. *The Lancet*, 352:1207-1212.

Meng, J., Zhao, S., Doyle, M.P., Mitchell, S.E., & Kresovich, S. (1997). A multiplex PCR for identifying Shiga-like toxin-producing *Escherichia coli* O157:H7. *Letters in Applied Microbiology*, 24:172-176.

Nataro, J.P. & Kaper, J.B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*, 11:142-201.

Olsvik, O. & Strockbine, N.A. (1993). PCR detection of heat-stable, heat labile and shiga-like toxin genes in *E. coli*. In Persing, D. H., Smith, T. F., Tenover, F. C., and White, T. J. (Eds.). *Diagnostic Molecular Microbiology; Principles and application*. United States: Mayo Foundation. Pp.271-280.

Payne, J., Francis, C.M., & Philips, K. (1985). *A field guide to the mammals of Borneo*. Kota Kinabalu: The Sabah Society.

Peigne, C., Bidet, P., Mahjoub-Messai, F., Painvert, C., Barbe, V., Me'digue, C., Frapy, E., Nassif, X., Denamur, E., Bingen, E., & Bonacorsi, S. (2009). The Plasmid of *Escherichia coli* Strain S88 (O45:K1:H7) That Causes Neonatal Meningitis Is Closely Related to Avian Pathogenic *E. coli* Plasmids and Is Associated with High-Level Bacteremia in a Neonatal Rat Meningitis Model. *Infection and Immunity*, 77:2272-2284.

Radu, S., Ooi, W.L., Rusul, G., Karim, M.L.A., & Nishibuchi, M. (2001). Detection of *Escherichia coli* O157:H7 by multiplex PCR and their characterization by plasmid profiling, antimicrobial resistance, RAPD

and PFGE analyses. *Journal of Microbiological Methods*, 46:131-139.

Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M., Hargrett, N.T., Blake, P.A., & Cohen, M.L. (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine*, 308:681-685.

Santamaria, J. & Toranzos, G.A. (2003). Enteric pathogens and soil: a short review. *International Microbiology*, 6:5–9.

Wallace, J.S., Cheasty, T., & Jones, K. (1997). Isolation of Vero cytoxin-producing *Escherichia coli* O157 from wild birds. *Journal of Applied Microbiology*, 82:399-404.