Captive wildlife from India as carriers of Shiga toxin-producing, Enteropathogenic and Enterotoxigenic Escherichia coli

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ABSTRACT. Shiga toxin-producing Escherichia coli (STEC), Enteropathogenic E. coli (EPEC), and Enterotoxigenic E. coli (ETEC) make up an important group of pathogens causing major animal and public health concerns worldwide. The aim of this study was to determine the prevalence of different pathotypes of E. coli in captive wildlife. We analyzed 314 fresh fecal samples from captive wildlife, 30 stool swabs from animal caretakers, and 26 feed and water samples collected from various zoological gardens and enclosures in India for the isolation of E. coli, followed by pathotyping by multiplex PCR. The overall occurrence rate of E. coli was 74.05% (274/370). The 274 E. coli isolates were pathotyped by multiplex PCR targeting 6 genes. Of them, 5.83% were pathotyped as EPEC, 4.74% as STEC, and 1.09% as ETEC. The 16S rRNA genes from the selected isolates were amplified, sequenced, and a phylogenetic tree was constructed. The phylogenetic tree exhibited indiscriminate genetic profiling and some isolates from captive wild animals had 100% genetic identity with isolates from caretakers, suggesting that captive wildlife may serve as a reservoir for infection in humans and vice-versa. The present study demonstrates for the first time the prevalence of these E. coli pathotypes in captive wildlife in India. Our study suggests that atypical EPEC strains are more frequent than typical EPEC strains in captive wildlife. Discovering the implications of the prevalence of these pathotypes in wildlife conservation is a challenging topic to be addressed by further investigations.

KEY WORDS: captive wildlife, Enteropathogenic E. coli (EPEC), Enterotoxigenic E. coli (ETEC), India, shiga-toxin producing Escherichia coli (STEC)
enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and diffuse-adherent *E. coli* (DAEC) [5]. Although pathogenic *E. coli* is extensively studied in humans, farm animals, food, and the environment, it is not well studied in wildlife.

EPEC infection can result in attaching and effacing (A/E) lesions and EPEC strains can be categorized as either “typical” or “atypical” based on the presence or absence of *E. coli* adherence factor plasmids, which contain the cluster of genes encoding bundle-forming pili (bfp) [32]. Humans are the only known reservoir for typical EPEC strains, being carried by asymptomatic adults and both symptomatic and asymptomatic children [13]. Atypical strains have been isolated from both humans and animals, including sheep, dogs, rabbits and monkeys [20, 30]. Several human and animal EPEC strains are clonally related and share various virulence characteristics [20]. The outcome of STEC infection ranges from asymptomatic carriage to diarrhea to more severe symptoms including hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS). Owing to their human pathogenicity, some STEC strains are classified as enterohemorrhagic *E. coli* (EHEC). EHEC strains comprise a subgroup of STEC and are characterized by certain serotypes, which are frequently associated with outbreaks and severe clinical illnesses [22]. EHEC strains are currently considered to have evolved from EPEC strains through the acquisition of bacteriophages encoding *stx* [24, 34]. Ruminants, especially dairy and meat cattle are known to be the primary reservoirs for EHEC and exposure to their fecal matter constitutes an important source of human infection [9]. ETEC, which is endemic in most underdeveloped countries, is a major cause of traveler’s diarrhea, diarrhea in infants, and colibacillosis in calves and piglets [23]. Contaminated food and water and direct contact with an infected person or animal are the most common sources of infection [31]. ETEC toxins (LT & ST) are associated with ETEC strains and strains are typically produce either LT or ST or both LT & ST. Globally, 60% of isolates expressed LT either alone (27%) or in combination with ST (33%) [10]. In most developing countries, and especially in India, little is known about the presence of *E. coli* pathotypes in wildlife. With this in mind, the present study aimed to investigate the prevalence of STEC, EPEC, and ETEC in captive wild animals, their caretakers, and their feed and water in different regions across India and to decipher the phylogenetic relationships between the recovered isolates.

**MATERIALS AND METHODS**

**Study area**

The study was carried out in 4 zoological gardens and wildlife enclosures, viz., Kanpur Zoo, Kanpur, Uttar Pradesh; Nainital Zoo, Nainital, Uttarakhand; Deer Park, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh; and the Post Graduate Research Institute of Animal Sciences, Chennai, Tamil Nadu, India.

**Sample description**

A total of 370 samples were obtained, comprising the fresh fecal samples of 314 healthy captive animals (40 species), 30 stool swabs from animal caretakers, and 26 feed and water samples. To obtain fecal samples, approximately 10 g of fecal matter was collected from clinically healthy animal using gloves and Cary-Blair transport media (Himedia, Mumbai, India). Caretakers were given sterile swabs to collect stool samples. The samples were placed in a chilled box, transported to the laboratory, and processed immediately.

**Isolation, identification, and confirmation of *E. coli***

Approximately 1 g/ml of each sample (fecal pellet/feed/water) was suspended in 9 ml of MacConkey broth (Himedia, Mumbai, India) and incubated overnight at 37°C. After enrichment, 10 µl of the product was streaked onto cosin methylene blue (EMB) agar (Himedia) and incubated (37°C, 24 hr). Up to three dark colonies with a green metallic sheen were picked and separately sub-cultured on EMB agar for 24 hr at 37°C for purification. Purified strains were further identified using gram-staining and a HilMViC biochemical kit (Himedia). Isolates were grown on LB broth (Difco Labs, Detroit, MI, U.S.A.) for 18 hr, after which the genomic DNAs of all the isolates were extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). To confirm the isolates’ identities as *E. coli*, species-specific PCR was employed using a previously reported protocol [26].

**Pathotyping**

The DNA of all the confirmed *E. coli* isolates were further subjected to previously reported PCR protocols [33] for the identification of pathotypes. The targeted pathotypes (genes) were shiga toxin-producing (*stx*1, *stx*2, and *eae*), typical enteropathogenic (*eae* and *bfp*), atypical enteropathogenic (*eae*), and enterotoxigenic (*stII* and *lt*) *E. coli*. PCR primers specific to *stx*1 and *stx*2 have previously been described by Cebula et al. [4], and those specific to *eae*, *bfp*, *stII*, and *lt* have been described by Vidal et al. [33]. The diarrheagenic *E. coli* reference strains EH18D (*stx*1, *stx*2, *eae*), ET12C (*lt*), ET117C (*stII*), and EP72D (*eae* and *bfp*) from our laboratory were used as positive controls. The positive control DNAs were pooled for multiplex PCR analysis to detect virulence genes (*stx*1, *stx*2, *eae*, *bfp*, *stII*, and *lt*).

**Phylogenetic analysis**

We selected isolates from various sources irrespective of their pathotypes using a random number table generated in Microsoft Excel™, giving due weightage to the number of isolates from a particular source. We selected five isolates each from ruminants, non-ruminants, birds, and caretakers and three isolates from feed and water sources. The 16S rRNA genes of the selected 23 *E. coli* isolates from various sources were amplified by PCR [18]. Sequencing of the amplified 16S rRNA genes of the selected isolates was
outourced to Eurofin, Bangalore, India, to whom we provided purified PCR products obtained using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The obtained sequences were analyzed using Gene Tool, DNA Star, Chromas Lite, and MEGA version 6.0, in which multiple sequence alignment was performed and percent identities and phylogenetic trees were retrieved. The edited sequences were subjected to multiple alignment using ClustalW and a pairwise distance comparison was performed with bootstrapping (1,000 replications) throughout the analysis and a neighbor-joining (NJ) phylogenetic tree was constructed using the distance algorithms in the MEGA6 package. The nucleotide sequences were deposited in GenBank using the National Centre for Biotechnology Information (NCBI, Bethesda, MD, U.S.A.) Bankit submission tool (http://www3.ncbi.nlm.nih.gov).

RESULTS

Microbiological, biochemical and PCR analysis of the 370 samples resulted in 274 (74.05%) positive identifications. A total of 93 isolates from captive wild ruminants (n=126), 67 isolates from captive wild non-ruminants (n=86), 79 isolates from captive wild birds (n=102), 24 isolates from caretakers’ stool samples (n=30), and 11 isolates from feed and water samples from wildlife enclosures (n=26) were identified and confirmed as E. coli.

Pathotyping using multiplex PCR analysis of the confirmed E. coli isolates provided the following results. A total of 32 (11.67%) isolates were successfully pathotyped. Among these 32 E. coli isolates, EPEC was found to be the predominant pathotype with an isolation rate of 5.83% (16/274), followed by STEC (4.7%, 13/274), and ETEC (1.09%, 3/274). Among the 13 isolates identified as STEC, 3 (23%) carried only stx1, 5 (38.4%) only stx2, 3 (23%) carried both stx1 and stx2, and 2 (15.4%) carried the stx1 and stx2 genes as well as the eae gene. Of the 16 isolates identified as EPEC, 14 (87.5%) were atypical, bearing only the eae gene, and 2 (12.5%) were typical, bearing both the eae and bfp genes. Of the 3 isolates identified as ETEC, 2 (66.7%) carried only the lt gene and 1 (33.3%) carried only the stII gene. Among EPEC pathotypes, the highest isolation rate (7.84%, 8/102) was observed in captive wild birds, followed by captive wild non-ruminants (5.81%, 5/86) and captive wild ruminants (1.58%, 2/126). Among the 8 isolates recovered from captive wild birds, 7 were found to be atypical EPEC strains, carrying only the eae gene, with one isolate recovered from ostriches being a typical EPEC strain bearing both the eae and bfp genes. Among the 5 isolates recovered from captive wild non-ruminants, all were found to be atypical EPEC strains carrying only the eae gene. Both the isolates recovered from captive wild ruminants were also found to be atypical EPEC strains carrying only the eae gene. Among birds, ostriches were found to be the most likely to harbor EPEC pathotypes with an isolation rate of 14.28% (5/35). Among the 5 EPEC isolates recovered from ostriches, one was found to be a typical EPEC strain carrying both the eae and bfp genes and the remaining four were atypical EPEC strains carrying only the eae gene. Interestingly, one typical EPEC isolate carrying both the eae and bfp genes was also detected in the ostriches’ caretaker. Among STEC pathotypes, the highest isolation rate (7.14%, 9/126) was observed among captive wild ruminants, especially in deer and antelopes. The next-highest isolation rate was in non-ruminants (3.48%, 3/86), while no STEC pathotypes were found in the sampled birds. Among the 9 STEC isolates recovered from captive wild ruminants, 2 carried all three genes (stx1, stx2, and eae), 2 isolates carried both the stx1 and stx2 genes, 3 isolates carried only the stx2 gene, and the other 2 isolates carried only the stx1 gene. Among ETEC pathotypes, the highest isolation rate was in human caretakers (6.7%, 2/30), with recovered isolates carrying the lt gene. One ETEC isolate was recovered from black buck (1/17, 5.88%) which carried the stII gene. One typical EPEC isolate (1/30, 3.33%) carrying both the eae and bfp genes was isolated from the stool of the ostriches’ caretaker and two ETEC (2/30, 6.7%) isolates carrying the lt gene were recovered from two caretakers at Kanpur zoo. One STEC carrying both stx1 and stx2 was isolated from feed and water samples (1/26, 3.84%). Species-specific prevalences are listed in Tables 1–3.

The 16S rRNA genes of 23 E. coli isolates recovered from a diverse array of sources were amplified, sequenced and submitted to GenBank (see Table 4 for additional information including accession numbers). Of the 23 isolates, 20 isolates were grouped into the same cluster (except for IHD4, NCT5, and KHY5) within which KBB6, KW1, and NL2 formed a separate subcluster, and KHY5 formed a subcluster with previously submitted sequences from the U.S.A., the U.K., and Japan (Fig. 1). Percent identity and divergence determined by analyses using MEGALIGN in DNA star revealed 80.2–100% identity and a divergence of 0.0–3.2 between the isolates. Four E. coli isolates from captive wild ruminants (KTD3), captive wild non-ruminants (KJ2), captive wild birds (KSCR2), and animal caretakers (KCT4) from Kanpur zoo exhibited 100% identity. Two E. coli isolates, viz., KW1 and KBB6 also exhibited 100% identity. Three E. coli isolates from Nainital zoo recovered from a captive wild ruminant (NBD2), a captive wild non-ruminant (NJM1), and an animal caretaker (NCT2) showed 100% identity.

DISCUSSION

This study is the first report of EPEC and ETEC strains in zoos in India. Of the 274 tested E. coli isolates, 32 were pathotyped as either STEC, EPEC, or ETEC. Others (232) did not belong to these pathotypes and may be commensal or could belong to other pathotypes not tested for in this study. The lack of studies on E. coli pathotypes in captive wildlife is primarily due to the difficulty associated with obtaining samples. The only other study which investigated a similar issue in India evaluated the prevalence of VTEC in deer, reporting an overall prevalence of 9.37% and the prevalence of strains carrying stx1 (26.6%), stx2 (33.3%), and both stx1 and stx2 (20%) [19]. The prevalences of isolates carrying either stx1 or stx2 or both stx1 and stx2 reported in this study are similar to those reported by this study [19]. The prevalence of the stx1 gene (24%) reported by another study carried out in Belgium is concordant with our findings [2]. However, higher stx2 prevalences exceeding 65% have also been reported by prior studies [28, 29]. The prevalence of the possession of both stx1 and stx2 according to a prior study is 28.6% [7], slightly higher than the values reported here. In our
study, 2 isolates of what is likely EHEC carried both stx and eae [24, 34]. Another study conducted in Argentina found similar results to those reported here, finding prevalences of isolates carrying stx1 (20%), stx2 (15.4%), and both stx1 and stx2 (15.4%) [12]. To the best of our knowledge based on the literature reviewed, this is the first report of STEC in thamin deer, barking deer, nilgai, Himalayan goral, leopard, and jackal. However, STEC has previously been reported in spotted deer and blackbuck [12].

The prevalence of EPEC in our study was 5.83%. In a study of wild cervids in Belgium, 1.5% (6/399) of EPEC isolates carried only the eae gene [2], lower than in our study. Our study is also concordant with a previous study of captive psittacines in Brazil, where 6.52% (3/46) of typical EPEC isolates carried both the eae and bfp genes [27]. In our study, the prevalence of EPEC among monkeys was 13.3% (2/15). Isolations of EPEC from rhesus and bonnet macaque simian immunodeficiency virus-infected infants and adult rhesus macaques has also been reported [15]. In another study carried out in healthy monkeys, the prevalence of EPEC was 26%, which is higher than our observations [3]. Similar to other studies, atypical EPEC strains were found to be more prevalent than typical strains [2, 11], suggesting that atypical EPEC strains are becoming increasingly more frequent relative to typical EPEC strains. Despite the fact that humans are the only known reservoir for typical EPEC strains [13], we were able to isolate typical EPEC from ostriches and from one of their caretakers. Earlier studies also reported typical EPEC in captive psittacines [27] and cats [8]. Our present findings may be valuable in emphasizing that animal caretakers and visitors may serve as both sources and reservoirs in the infection of captive animals by EPEC and vice versa. Thus, we hypothesize that animals which test positive for

| Table 1. Prevalence of E. coli and its pathotypes (STEC, EPEC, and ETEC) in fecal samples collected from captive wild ruminants |
|-----------------------------------------------|----------------|------------------|----------------|------------------|------------------|-----------------|------------------|
| Common name                           | Scientific name | No. of samples | E. coli Pathotypes | STEC | EPEC Typical | EPEC Atypical | ETEC |
|-----------------------------------------------|----------------|------------------|----------------|------------------|------------------|-----------------|------------------|
| Sambar deer                            | Rusa unicolor  | 5                | 5               | –                | –                | –               | –                |
| Himalayan goral                        | Naemorhedus goral | 8            | 5               | 2                | –                | –               | –                |
| Barking deer                           | Muntiacus muntjak  | 5             | 3               | 1                | –                | –               | –                |
| Thamin deer                            | Panolia eldii   | 10             | 7               | 1                | –                | –               | –                |
| Swamp deer                             | Cervus davaucelli | 15           | 11              | –                | –                | 1               | –                |
| Nilgai                                | Boselaphus tragocamels | 12      | 8               | 1                | –                | –               | –                |
| Spotted deer                           | Axis axis       | 32             | 25              | 3                | –                | –               | –                |
| Blackbuck                              | Antilope cervicapra | 17         | 13              | 1                | –                | –               | 1                |
| Indian hog deer                        | Cervus porcinus  | 15            | 12              | –                | –                | 1               | –                |
| Sika deer                              | Cervus Nippon   | 3              | 1               | –                | –                | –               | –                |
| Chousinga deer                         | Tetracerus quadricornis | 2         | 2               | –                | –                | –               | –                |
| Himalayan blue sheep                   | Pseudois nayar  | 2              | 1               | –                | –                | –               | –                |
| Total                                  |                | 126            | 93              | 9                | –                | 2               | 1                |

| Table 2. Prevalence of E. coli and its pathotypes (STEC, EPEC and ETEC) in fecal samples collected from captive wild non-ruminants |
|-----------------------------------------------|----------------|------------------|----------------|------------------|------------------|-----------------|------------------|
| Common name                           | Scientific name | No. of samples | E. coli Pathotypes | STEC | EPEC Typical | EPEC Atypical | ETEC |
|-----------------------------------------------|----------------|------------------|----------------|------------------|------------------|-----------------|------------------|
| Leopard                                | Panthera pardus  | 20             | 16              | 1                | –                | 1               | –                |
| Bengal Tiger (inc. one white)             | Panthera tigris tigris | 9          | 7               | –                | –                | 1               | –                |
| Hyena (striped)                         | Hyaena hyaena   | 10             | 8               | 1                | –                | –               | –                |
| Tibetan wolf                           | Canis lupus chanco | 2           | 2               | –                | –                | –               | –                |
| Jackal                                 | Canis aureus    | 5              | 4               | 1                | –                | –               | –                |
| Himalayan black bear                    | Ursus thibetanus laniger | 7        | 5               | –                | –                | 1               | –                |
| Sloth bear                             | Melursus ursinus | 2            | 2               | –                | –                | –               | –                |
| Hippopotamus                           | Hippopotamus amphibious | 6        | 4               | –                | –                | –               | –                |
| Indian rhinoceros                      | Rhinoceros unicornis  | 3          | 2               | –                | –                | –               | –                |
| Gray langur                            | Semnopithecus entellus  | 5          | 5               | –                | –                | –               | –                |
| Bonnet macaque                         | Macaca radiate  | 5              | 5               | –                | –                | 1               | –                |
| Rhesus macaque                         | Macaca mulatta  | 3              | 3               | –                | –                | 1               | –                |
| Japanese macaque                       | Macaca fuscata  | 2              | 2               | –                | –                | –               | –                |
| Palm civet                             | Paradoxurus hermaphroditus | 3        | –               | –                | –                | –               | –                |
| Red panda                              | Ailurus fulgens  | 2              | 1               | –                | –                | –               | –                |
| Leopard cat                            | Prionailurus bengalensis | 1        | 1               | –                | –                | –               | –                |
| Zebra                                  | Equus quagga    | 1              | –               | –                | –                | –               | –                |
| Total                                  |                | 86             | 67              | 3                | –                | 5               | –                |

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A common human pathogen [22], could be zoonotic sources of infection which were initially infected following direct or indirect contact with humans and domestic animals. To the best of our knowledge, based on the literature reviewed, this is the first report of EPEC in leopard, tiger, Himalayan bear, grey langur, bonnet macaque, swamp deer, hog deer, Indian peafowl, and saras crane.

In total, only 3 E. coli isolates were identified as ETEC. Two isolates were from caretakers and carried only the lt gene and one isolate was recovered from blackbuck which carried the stII gene (5.88%). The true prevalence of ETEC is unknown due to the scarcity of studies in this area of disease biology. ETEC is a very common pathogen infecting piglets, calves, and travelers and is also thought to infect other important farm animals like horses, rabbits, and poultry, as these animals possess the ability to detect and respond to ETEC enterotoxins and adhesins [21]. While this is the first report of ETEC in blackbuck, a previous study carried out in China reported that E. coli isolated from farm-raised sika deer carried both the lt and st genes at a frequency of 6.8% [14].

Using 16S rRNA gene sequencing analysis, we determined that clustering was independent of pathotype. Notably, three different pathotypes (NL2-atypical EPEC, KW1- STEC, and KBB6-ETEC) clustered together. 16S rRNA sequencing analysis was found to be unsatisfactory in the monitoring of the relationships among strains. We suggest that pulsed-field gel electrophoresis (PFGE) or multi-

### Table 3. Prevalence of E. coli and its pathotypes (STEC, EPEC and ETEC) in fecal samples collected from captive wild birds

| Common name          | Scientific name          | No. of samples | E. coli Pathotypes |
|----------------------|--------------------------|----------------|-------------------|
| Golden pheasant      | Chrysolophus pictus      | 8              | 7                 | –                 | –                 | –                 |
| Silver pheasant      | Lophura nycthemera       | 8              | 6                 | –                 | –                 | –                 |
| Cockatiel            | Nycticola hollandicus    | 6              | 5                 | –                 | –                 | –                 |
| Lady Amherest Pheasant| Chrysolophus amherstiae  | 12             | 9                 | –                 | 1                 | –                 |
| Kali pheasant        | Lophura leucocomelans    | 8              | 6                 | –                 | –                 | –                 |
| Sun conure           | Aratinga solstitialis    | 6              | 3                 | –                 | –                 | –                 |
| Red Jungle fowl      | Gallus gallus            | 2              | 2                 | –                 | –                 | –                 |
| Indian peafowl       | Pavo cristatus           | 4              | 3                 | –                 | 1                 | –                 |
| White peafowl        | Pavo cristatus mut. alba | 3              | 3                 | –                 | –                 | –                 |
| Saras crane          | Grus antigone            | 5              | 4                 | –                 | –                 | 1                 |
| Emu                  | Dromaius novaehollandiae | 5              | 4                 | –                 | –                 | –                 |
| Ostrich              | Struthio camelus         | 35             | 27                | –                 | 1                 | 4                 |
| **Total**            |                          | **102**        | **79**            | **1**             | **7**             | **–**             |

### Table 4. Isolate details and their accession numbers

| S. No. | Accession numbers | Isolate No. | Pathotype | Species and place of sampling |
|--------|-------------------|-------------|-----------|-----------------------------|
| 1      | KT005220          | NJF1        | –         | Jungle fowl, Nainital       |
| 2      | KT005221          | NCT2        | –         | Caretaker, Nainital         |
| 3      | KT005222          | NGP4        | –         | Golden pheasant, Nainital   |
| 4      | KT005223          | NW2         | –         | Water, Nainital             |
| 5      | KT005224          | KCOK4       | –         | Cockatiel, Nainital         |
| 6      | KT005225          | NCT5        | –         | Caretaker, Nainital         |
| 7      | KT005226          | IHD4        | Atypical EPEC | Hog deer, IVRI          |
| 8      | KT005227          | KBB6        | ETEC      | Black buck, Kanpur          |
| 9      | KT005228          | NBD2        | STEC      | Barking deer, Nainital      |
| 10     | KT005229          | NL2         | Atypical EPEC | Leopard, Nainital        |
| 11     | KT005230          | KSCR2       | Atypical EPEC | Saras crane, Kanpur      |
| 12     | KT005231          | KW1         | STEC      | Water, Kanpur               |
| 13     | KT005232          | PGRIASCT2   | Typical EPEC | Caretaker, PGRIAS   |
| 14     | KT005233          | IW1         | –         | Water, IVRI                |
| 15     | KT005234          | NJM1        | –         | Japanese macaque, Nainital  |
| 16     | KT005235          | ICT1        | –         | Caretaker, IVRI             |
| 17     | KT005236          | KJ2         | STEC      | Jackal, Kanpur              |
| 18     | KT005237          | KN5         | STEC      | Nilgai, Kanpur              |
| 19     | KT005238          | KTD3        | STEC      | Tamin deer, Kanpur          |
| 20     | KT005239          | KRZ1        | –         | Rhinoceros, Kanpur          |
| 21     | KT005240          | KHY5        | STEC      | Hyena, Kanpur               |
| 22     | KT005241          | PGRIASO5    | Typical EPEC | Ostrich, PGRIAS   |
| 23     | KT005242          | KCT4        | ETEC      | Caretaker, Kanpur           |

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locus sequence typing should be used to determine valid genetic variation among strains. However, our 16S rRNA gene sequencing analysis did reveal indiscriminate genetic profiling among the isolates of different animal species and their caretakers. The isolation of similar pathotypes (typical EPEC) from an ostrich (PGRIAS05) and its caretaker (PGRIASCT2) suggests the circulation of similar clones among captive wildlife and their caretakers. Thus, we suggest that as similar clones are circulated and maintained within captive wildlife, their caretakers, and their water sources, wildlife may serve as a reservoir for infection in humans and vice versa.

In conclusion, our study suggests that atypical EPEC strains are becoming more frequent than typical EPEC strains in captive wildlife, signifying that atypical EPEC strains have the potential to be a zoonotic pathogen. We observed a high frequency of STEC infection in captive wildlife and suggest that some measures to reduce the risk of exposure of caretakers and visitors at zoos should be implemented, as young children commonly visit zoos and other animal parks and are thus likely to become infected and potentially suffer severe complications such as HUS.

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