

Do camels (*Camelus dromedarius*) play an epidemiological role in the spread of Shiga Toxin producing *Escherichia coli* (STEC) infection?

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Accepted: 5 December 2007 / Published online: 19 December 2007
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Abstract In the present work, faecal and serum samples from 400 camels were investigated for the presence of Shiga Toxin producing *E.coli* (STEC) and Anti-Shiga Toxin (Anti-Stx) antibodies, respectively. The used samples were obtained from adult male camels of five east African countries (Egypt, Somalia, Djibouti, Kenya and Sudan) between the years 2002–2004. One *E.coli* isolate per camel was randomly selected to be cultured on Gassner, Chromocult and sorbit agar for the detection of O157:H7 strains. In the same time, a Stx-specific PCR screening was performed for the isolates using the shiga toxin specific primers Mk1-Mk2. Vero cells were also used for shiga toxin neutralization assay. None of the investigated isolates reacted positively with the Stx-specific primers. Also, none of the studied sera could neutralize the Stx on tissue culture. The obtained results indicate that camels do not play any significant epidemiological role in STEC infection and transmission. The possible reasons for the absence of STEC in the investigated samples are discussed in brief.

Keywords Camelus dromedarius · Djibouti · Egypt · Kenya · STEC · Somalia · Sudan

Abbreviations

CPE Cytopathic Effect
STEC Shiga Toxin producing *E.coli*
Stx Shiga Toxin

Introduction

Like many bacterial virulence factors the shiga toxin encoding genes are carried on bacteriophages. Permanent phage evolution occurs through phage recombination, leading to the development of new phage or toxin variants. The evolved phages differ in their genetic structure (El-Sayed 2000; Herold et al. 2004) enabling them to infect new bacterial strains and possibly new animal hosts. The STEC are responsible for many serious, even fatal, diseases in humans and animals. They were isolated from different mammals, bird and insect species but ruminants were found to be the main reservoir, particularly for *E.coli* serogroup O157. In ruminants STEC were isolated from cattle, buffaloes, sheep, goat, deer and other wild ruminants (Beutin et al. 1993; Zschoeck et al. 1998; Nielsen et al. 2004). However with the exception of Moore et al. (2002) no data are available about the prevalence of STEC in camels. STEC from this origin would have an epidemiological importance due to the free

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movement of camel herds through the desert spreading infection from one country to another (El-Sayed 1996). Most studies on STEC have focused on the serogroup O157: H7 due to its major importance for the public health. In the present work the prevalence of STEC, especially O157:H7 strains, in camels was investigated biochemically, molecular biologically and serologically.

Materials and methods

Sampling and bacterial characterization

Between 2002 and 2004, faecal and serum samples were collected from 400 adult male camels from five African countries; namely, Egypt, Somalia, Djibouti, Kenya and Sudan; eighty camels each. The faecal samples were transported in LB media to the laboratory where they were cultured on Gassner's Agar. Five lactose positive colonies per animal were selected for further biochemical examinations including Indol and H₂S production, citrate and urea hydrolysis and glucose fermentation tests. One *E. coli* strain per animal was selected randomly for further investigation. All 400 *E. coli* strains were re-cultured on Chromocult and sorbit agar for the screening of O157 strains (El-Sayed 2000). The *E. coli* reference strain K12 (C600) was used as a negative control while the reference strains 537/89 (O84:H-, *stx1*) and RW 0304 (O145:H28, *stx2*) were used as positive control for the PCR and toxin production.

Molecular biological screening

The strains were investigated by *stx*- specific PCR using the previously described universal primer MK1-MK2 (Karch and Meyer 1989). Briefly, the PCR was performed in a total volume of 50 µl containing 5 µl of 10-fold PCR buffer, 5 µl of MgCl₂ (25 mM), 2.5 µl of each primer (10 µM), 1 µl of deoxynucleoside triphosphate (10 mM each nucleotide), and 1 µl of Taq polymerase (2.5 U/µl; ABgene, Hamburg, Germany). The amplification of a 230 bp fragment from the *stxA* unit of the shiga toxin encoding gene was carried out using the oligonucleotide primer MK1 (5'-TTT ACG ATA GAC TTC TCG AC- 3') and MK2 (5'- CAC ATA TAA ATT ATT TCG CTC- 3') supplied by MWG Biotech (Ebersberg, Germany) and the following thermocycler program: 1x denaturation at 94°C for

180 s, followed by 30 times 94°C - 60 s, 44°C - 60 s, 72°C - 90 s, and a final cycle at 72°C for 5 min. then cooling at 4°C. Later on, 10 µl of the amplificate was examined by gel electrophoresis on 1% agarose gel and stained with ethidium bromide dye.

Toxin purification

Crude Stx was prepared from the control STEC strains 537/89 (O84:H-, *stx1*) and RW 0304 (O145:H28, *stx2*). The investigated bacteria were grown for 12 h at 37°C in 8 l minimal essential medium (3.5 g K₂HPO₄, 1.5 g KH₂PO₄, 0.5 g sodium citrate dihydrate, 0.1 g MgSO₄×7 H₂O, 1.0 g (NH₄)₂SO₄, 2.0 g glucose ad 1 l distilled water). The bacteria were harvested by centrifugation and sonicated. Supernatant was clarified by ultracentrifugation (100,000×g, 2.5 h) and diluted 1:1 with 10 mM sodium phosphate buffer (pH 7.4).

Standard Stx neutralization assay

The test was performed on Vero cells (ATCC CRL 1587) as described previously (Pirro et al. 1995; El-Sayed 1996). Briefly, the 50% cytotoxic dose (CD₅₀) was determined in 96 well microtiter plates (Nunc, Wiesbaden, Germany) by incubating Vero Cells with 90 µl/ well of the logarithmic dilutions of Stx1 or Stx2 solution. CD₅₀ was recorded as the reciprocal of the highest dilution causing cytotoxic effects in 50% of the cells after 96 h of incubation. The Stx neutralization activity of sera (1:10) was determined by the incubation of 10 µl of diluted sera with 90 µl/ well of the logarithmic dilutions of Stx1 or Stx2 solution for 1 h at 37°C. Then 5×10⁴ Vero cells were added per well and the plates were incubated for 96 h at 37°C in 5% CO₂. On each plate, controls including Vero Cells and Vero cells incubated with either monoclonal anti-Stx1 (13C4) or anti-Stx2 (11F11) antibodies were performed as negative and positive controls, respectively. In addition, Vero Cells were directly incubated with the samples to exclude their non specific toxic effect on the cells. The Vero Cells were examined microscopically for the presence of Stx-induced cytopathic effect (CPE).

Results

In the present study faecal and serum samples from 400 male camels were obtained from Egypt, Somalia,

Djibouti, Kenya and Sudan. None of the investigated strains showed the expected biochemical profile of O157:H7 by the use of the Chromocult and sorbit agar. With the exception of the positive control strains, none of the investigated isolates reacted positively for the *stx* specific PCR (Fig. 1). All the performed bacteriological, molecular biological and serological examinations revealed the absence of STEC strains and detectable anti-Stx antibodies among the 400 investigated samples.

Discussion

Between the year 2002 and 2004, faecal and serum samples were collected from camels of five east African countries. The samples were subjected to different bacteriological, molecular biological and serological examinations. The obtained results revealed the absence of STEC strains and anti-Stx antibodies among the investigated samples. As the *E. coli* O157 strains are difficult to be detected in studies targeting all STECs due to lower sensitivity (Lahti 2003), additional biochemical screening for O157:H7 was performed in the present work using Chromocult and sorbit agar (El-Sayed 2000). The absence of O157:H7 in camels was recorded previously by Moore et al. (2002) who investigated faecal samples from 67 camels in the United Arab Emirate for this purpose. The presence of other STEC strains was not investigated by this team. The present study investigated a larger number of camels, from 5 countries and with additional serological confirmation for the

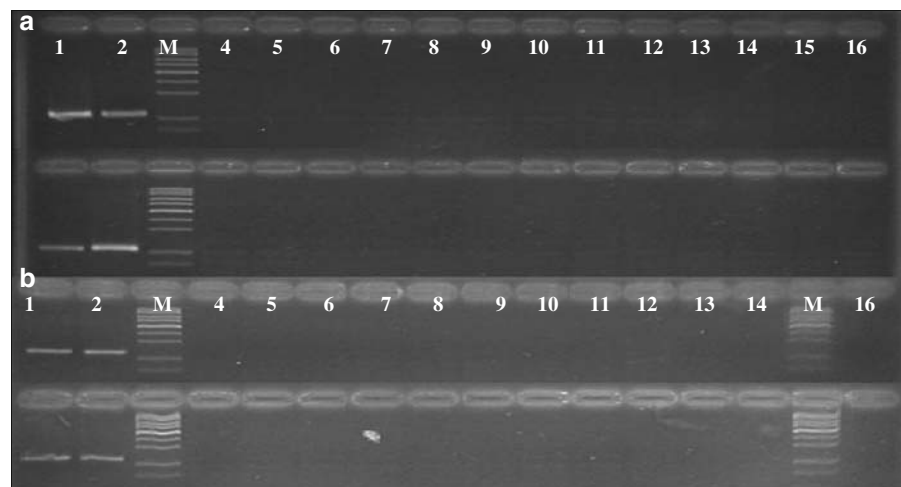
presence of STEC. The absence of STEC among camel isolates may be attributed to one or more of the following reasons:

- (1) Camel STEC variants can not be detected by the universal primer MK1-Mk2:

A similar condition was previously described concerning the isolation of STEC strains from pigeons. The *stx2f* could not be detected by the universal primer MK1-MK2 due to sequence deviation at the primer attachment site (Schmidt et al. 2000). To exclude this possibility in the present work, Stx production was investigated by serum neutralization test on cell culture. The occurrence of the characteristic cytopathic effect on the Vero cells indicated the absence of neutralizing anti-Stx antibodies in the tested sera.

- (2) Camels are resistant to STEC infection: As the rumen flora in camels differ from other ruminants; this, in turn, may influence the micro environment of STEC. Although the O157: H7 strains and the encoded *stx*-variants are relatively host specific (Kim et al. 1999), their wide range of susceptible animal and insect species indicates the absence of string host specificity. This possibility seems to be improbable.
- (3) STEC infection is not (yet) widespread among camels and consequently difficult to detect. This hypothesis can be excluded or confirmed only through the examination of a larger number of camel *E. coli* isolates from endemic countries, from zoo-camels and from camel herds not living in the desert.

Fig. 1 The figure presents the PCR screening for STEC strains. Line 1 (strain 537/89 as Stx1 positive control), Line 2 (strain RW 0304 as Stx2 positive control), Line 16 (strain K12 as a negative control strain) and M (100 bp marker, New England Biolabs, Germany). The lines 4–15 (a) and 4–14 (b) represent a part of the investigated camel isolates



- (4) The free life style of camels: STEC are mostly detected in developed countries where large animal farms are common. The free life style in general decreases the shedding of STEC in faeces in comparison with housed animals or those fed in pen (Synge et al. 2001; Gannon et al. 2002). In addition, the free life pattern of camels in the desert minimizes the contact of camels with other animal species and therefore the transmission of STEC to camels. The continuous evolution of new *stx*-encoding phages occurs where *E.coli* strains come in close contact with free phage particles such as in sewage or in contaminated rivers (Muniesa and Jofre 2004). Phage recombination leads to the evolution of new members through inter- and intra-species transmission. Different lines of evolution of *stx*-encoding phages are expected to exist in different countries and in different animal species. This possibility is supported by the results of antibiotic sensitivity test where the investigated strains were compared with strains isolated from cattle and pigs. Camel isolates were obviously more sensitive to antibiotics than isolates from other species (El-Sayed *et al.* unpublished data). The limited contact of camel strains with other *E.coli* strains lead to the delay in the transmission of mobile virulent factor encoding genes such as *stx* and the antibiotic resistance genes to camel isolates.
- (5) Diet and dietary stress in the desert: The nature of diet and its protein / fibre contents affect the colonization, shedding and clearance of STEC from the GIT. Diet low in nutrients and high in fibres enhances the shedding of STEC in faeces but induces in the same time the elimination of O157 strains from the GIT (Kudva et al. 1995). It is also reported that O157 can proliferate to high population in moist ration and moist silage and therefore infect new animals (Lynn et al. 1998; Sanchez et al. 2002). In the desert the dry and poor ration available for camels does not offer such a suitable environment. Camels starve for a long period and this disturbs the ecological system in their digestive system and offers unfavourable conditions for STEC.
- (6) Environmental and climatic factors: Although *E. coli* O157 can persist for a long time in the environment (Fukushima et al. 1999), it is

negatively influenced by heat and dryness under the desert conditions. Meanwhile, sand was proven to reduce the prevalence of STEC (Lejeune and Kauffman 2005). It is also possible that the very low water content in camel faeces decreases the survival chances of STEC under the undesirable environmental conditions. This in turn limits the horizontal transmission of STEC among camels.

In conclusion, the data obtained from this investigation refer to the absence of STEC infection in camels. This may be attributed to factors related to the STEC, the environment or to the camels themselves.

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