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# Type 2 heat-labile enterotoxin (LT-II)-producing *Escherichia coli* isolated from ostriches with diarrhea

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**Abstract**

The culture supernatant of *Escherichia coli*, isolated from ostriches with diarrhea in Brazil, caused elongation in Vero cell, rounding in Chinese hamster ovary (CHO) cells and a cytoplasmic vacuolation in ostrich embryo fibroblasts (OEF), but it was not cytotoxic for chicken embryo fibroblasts (CEF). These effects were not neutralized by antiserum to cholera toxin. Polymerase chain reaction assays showed that the ostrich *E. coli* contained the gene encoding (*elt*II-A), but not those for type 1 heat-labile enterotoxin (*eltA*), heat-stable enterotoxins (*estA*, *estB*), verocytotoxins (*stx*-I, *stx*-II), or cytotoxic necrotizing factors (*cnf* 1, *cnf* 2). All isolates belonged to serotype O15:H8. The enteropathogenic relevance of LT-II in ostrich diarrhea remains undetermined.


*Keywords:* *Escherichia coli*; Type 2 heat-labile enterotoxin (LT-II); Ostrich; Diarrhea; Cytoplasmic vacuolation

## 1. Introduction

The ostrich (*Struthio camelus australis*) is bred in many countries as an alternative meat source for human consumption and for the production of leather and plumes (El-Attrache et al., 2001; Knöbl et al., 2001; Ley et al., 2001). Although the commercial production of ostriches is widespread, little research has been undertaken on the occurrence of intestinal pathogens, such as *Escherichia coli* and *Salmonella* sp., in these birds (Ley et al., 2001).

Enterotoxigenic *E. coli* (ETEC) strains cause watery diarrhea in animals worldwide (Neill et al.,

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1994), and belongs to one of two groups: the heat-stable enterotoxins (STs) (Smith and Halls, 1967) and the heat-labile enterotoxins (LT-I and LT-II) (Green et al., 1983).

ETEC producing LT-I are usually associated with diarrhea in humans and piglets (Holmes et al., 1985; Osek et al., 1999); however, they have also been isolated from chickens (Tsuji et al., 1988), turkeys (Emery et al., 1992) and ostriches (Deng et al., 1996). ETEC producing LT-II are usually isolated from bovine sources (Holmes et al., 1985; Seriwatana et al., 1988; Osek et al., 1999; Ugrinovich et al., 2002). The objective of this study was to determine the presence of ETEC producing LT-II from feces of ostriches with diarrhea.

## 2. Materials, methods, results and discussion

### 2.1. Bacterial strains

Twenty-four 3-month-old ostriches, from a farm in São Paulo State (Brazil), showed diarrhea and died suddenly. Fecal samples were collected from 18 birds, on the same day, before they also die. These samples were cultured in brain heart infusion broth (BHI; Difco, USA), and then on MacConkey agar (Oxoid, UK). More than five colonies from each sample were isolated.

All animals were handled humanely, according to protocols approved by the Animal Care and Use Committee of the Universidade Estadual de Campinas (UNICAMP).

*E. coli* was identified using the media EPM, MILi (Toledo et al., 1982a,b), and Simmons citrate agar (Difco, USA).

The following bacterial strains were used as positive controls: bovine *E. coli* B62 (LT-II) (Penteado et al., 2002), *E. coli* O157:H7 (Stx1), kindly supplied by Dr. J.M. Lord (University of Warwick, UK), *E. coli* J-2 (Stx2), kindly supplied by Dr. Y. Takeda (Jiisen Unversity, Tokyo, Japan), *E. coli* 40T (LT-I), kindly supplied by L.R. Trabulsi (Instituto Butantan, SP, Brazil). *E. coli* C600 was used as a negative control.

*E. coli* strains were isolated from four samples, corresponding to 22% of the 18 ostriches. Other etiologies were also found: 11 isolates of *Serratia* sp., three isolates of *Salmonella* sp. and one isolate of *Enterobacter* sp. One sample showed two isolates: *Serratia* sp. and *Salmonella* sp.

### 2.2. Bacterial culture and toxin preparation

Bacteria strains were cultivated as reported Blanco et al. (1997a).

### 2.3. Cell culture lines

Chinese hamster ovary (CHO), African green monkey kidney (Vero), and chicken embryo related (CER) cells, kindly supplied by Dr. H.M. Hafez (Free University of Berlin, Germany), were grown at 37 °C in Eagle's minimum essential medium (EMEM) (Cultilab, SP, Brazil) containing 10% fetal calf serum (FCS) (Sigma, St. Louis, MO, USA), penicillin (1000 U/ml) and streptomycin (250 μg/ml).

### 2.4. Primary cultures of chicken and ostrich embryo fibroblasts

Ostrich embryo fibroblasts (OEF) were prepared as described by Freshney (2000), except that the embryos were used after 20 days of incubation. The chicken embryo fibroblasts (CEF) were kindly provided by Fort Dodge Laboratories (Campinas, SP, Brazil).

### 2.5. Cytotoxicity assay

Cytotoxic activity was detected as reported by Konowalchuk et al. (1977). The negative controls were EMEM, TSB containing mitomycin C and supernatant from a culture of *E. coli* C600. The heat stability of the toxins was assessed by heating the samples at 100 °C for 30 min.

The culture supernatants showed cytotoxic activity (elongation) on CHO and Vero cells, except for one isolate (Table 1) that had no effect on CHO cells, despite a titre of 1/16 on Vero cells.

Although, ostrich *E. coli* supernatants did not affect CEF or CER cells, they produced cytoplasmic vacuolation in OEF after 24 h (Fig. 1A and B), and heated culture supernatants lost their cytotoxicity. Similar vacuolation of OEF was also seen with the culture supernatants of bovine *E. coli* B62 positive control for LT-II toxin. This finding suggests that LT-II caused cytoplasmic vacuolation in OEF.