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The Bacterial Causes of Camel-calf (*Camelus dromedarius*) Diarrhea in Eastern Sudan.

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ABSTRACT

Fecal samples were collected from diarrheic camel-calves owned by nomadic pastoralists in the Butana Region. They were examined for 5 types of bacteria reported to cause the diarrhea. Sixty nine *E. coli* (66%), 14 *Salmonella spp.* (13%) and 11 *Clostridium perfringens* (10%) isolates were obtained from the samples. However, neither *Campylobacter spp.* nor *Yersinia enterocolitiaca* were isolated. The *E. coli* probing revealed the presence of K88, F41 adhesion factors and StaP, STb, LT, SLTII enterotoxins, while a high molecular weight plasmid was evident in the plasmid analysis profile. Thirteen of the *Salmonella* isolates were serotyped using group and specific antisera to be identified as *Salmonella typhi* strain.

Key words: Diarrhea, Bacteria, Calf, Camel, Sudan.

INTRODUCTION

In the Eastern province of Sudan, camel-calf diarrhea affects about 33% of the off-spring causing 23% mortality and accounts for a noticeable reduction in calf variability, while several cases die leading to reduction of herd growth (Abbas and Musa, 1988). The enteropathogens most commonly found and extensively studied were enterotoxigenic and enteropathogenic *Escherichia coli* (ETEC and ETPC), *Salmonella spp*, rotavirus, coronavirus, cryptosporidium (Tzipori, 1981) and, more recently, *Clostridium difficle* (Hove *et al.*, 1996).

The objective of this study is to conduct a preliminary diagnosis of the bacterial agents involved in camel-calf diarrhea in the Eastern province of Sudan.

MATERIALS AND METHODS

Sampels collection

Camel-calves (*Camelus dromedarius*) aged three months or younger owned by nomadic pastoralists of different tribes in the Butana region, Eastern province of Sudan were used in this study. Fecal samples were collected from calves with clinical signs of diarrhea. These calves had not been treated with antibiotics during the diarrhea episode. A single specimen was obtained from each animal during the study period. Samples were usually collected during the first week of each month.

Examinations for bacteria

E. coli and *Salmonella spp.* were cultivated on MacConkey; *Campylobacter spp.* on *Campylobacter* blood-free agar base and Cefoperazone selective supplement; *Yersinia enterocolitica* on *Yersinia* selective medium and *Clostridium perfringens* on Shahidi and Ferguson medium.

Identification of bacteria

Identification of the isolates was performed according to conventional methods. *Salmonella* isolates were grouped by slide agglutination and serotyped using *Salmonella* O and H antisera. Suspected colonies were tested by slide agglutination using specific antisera. Characteristic black colonies of *Clostridium perfringens* on Shahidi and Ferguson medium were further confirmed by Nagler's reaction on Egg Yolk Agar.

Plasmid screening procedure

E. coli isolates were examined for the presence of extrachromosomal DNA using the method described by Kado and Liu (1981). The DNA samples were examined by agarose gel

electrophoresis. *E. coli DNA probes.* The gene probes were specific to the enterotoxins LT, StaP, STb and to the shiga-like toxins SLT-I, SLT-II and to the adhesions F41, K88 and K99. These were labeled by ^{32}P using the nick translation method.

E. coli colonies hybridization. A total of 29 isolates were analyzed by colony hybridization with various gene probes. Hybridization with ^{32}P labeled probes was performed as described by Chang *et al.*, 1991.

RESULTS AND DISCUSSION

The results of this study revealed the importance of *E. coli* and *Salmonella spp.* in causing diarrhea in young camel-calves. A similar finding was reported in a previous study performed by Romboli (1942) describing endemic *E. coli* infections in newborn camels which resembled the neonatal coliform septicaemia in other domestic animals.

The *E. coli* adhesion factors which have been reported in animals are K88 (Mooi, *et al.*, 1979), K99 (de Graaf and Klaasen, 1986), F41 (Moseley, *et al.*, 1986) and 987P (Schifferliv, *et al.*, 1990). However, only K88 and F41 were probed in *E. coli* from camel-calves in this study. These colonization factors are plasmid mediated. *E. coli* produces three enterotoxins: two are heat-stable STa (STaP, STaH) and STb (Alderete and Robinson, 1978), while the third is heat-labile (LT) (Kunkel and Robertson, 1979). STaP, STb and LT were reported in 8 isolates in this study (8/29). Enterohemorrhagic *E. coli* secretes shiga-like toxin I (SLT-I) and shiga-like toxin II (SLT-II) (Karmali, 1989). However, only SLT-I were detected in 3 isolates in this study (3/29). These isolates from the camel-calves may have contributed similarly to the diarrhea. Some of the *E. coli* isolated did not contain the plasmids which might have been lost on sub culturing, a phenomenon which is known to happen (Evans, *et al.*, 1977).

Salmonellosis in camels had been reported from Sudan (Currason, 1918), United States of America (Bruner and Moran, 1949) and from Somalia (Cheyne, *et al.*, 1977). In the latter it was reported to affect the suckling camel-calves. However, the isolation of *Salmonella typhi* from these fecal samples was not reported before in animals, but its presence probably is due to contamination of drinking water from human sources.

Furthermore, *Clostridium perfringens* was isolated before from camels with severe bloody diarrhea and was reported as the cause of death in camels (Ipatenko, 1974). This disease had occurred in acute and subacute forms in Mongolia.

Some of the *E. coli* isolates in this study contained different molecular weight plasmids. Thirteen isolated had molecular weight plasmids (> 23 Kb). It is known that these high molecular weight plasmids can code for an antibiotic resistance (Gross *et al.*, 1982). This may explain the failure of antibiotic treatment in controlling some of the diarrhea episodes in camel-calves.

IMPLICATIONS

In this study, enteropathogenic *E. coli* was isolated for the first time from camel-calves with diarrhea in the Sudan. Some of these isolates produced high molecular weight plasmids which could code for antibiotic resistance. Further studies are planned to test for the presence of antibiotic resistance among the *E. coli* strains. Other pathogenic bacteria isolated from camel-calves were *Salmonella spp.* and *Clostridium perfringens.*

REFERENCES

Abbas, B. and B. E. Musa. 1988. A rapid field survey of camel husbandry in the northern Butana. In Musa, B.E., Melaku, A. and Wilson, R. T. (eds.). Camel research paper from Sudan. International Livestock Center for Africa, Addis Ababa, Ethiopia, 1-12.

Alderete, J. F. and D. C. Robinson. 1978. Purification and chemical characterization of the heat-stable enterotoxin produced by porcine strains of enterotoxigenic *Escherichia coli.* Infection and Immunity. 19: 1021-1030.

Bruner, D. W. and A. B. Moran. 1949. *Salmonella* infections of domestic animals. Cornell Veterinarian, 39: 53-63.

Chang, Y. F., R. Young and D. K. Struck. 1991. *Actinobacillus pleuropneumoniae* hemolysin determinant: unlinked appCA

and appBD loci flanked by pseudogenes. Journal of Bacteriology, 173: 5151-5158.

Cheyne, I. A., R. G. Pegram and C. F. Cartwright. 1977. An outbreak of *Salmonellosis* in camels in the north-east of the Somali Democratic Republic. Tropical Animal Health and Production, 9: 238-240.

Currason, G. 1918. Une maladie due dromadarie analogue an faracin du boeuf. Bulletin De La Societe De Mediche Veterinariae, 1: 491-496.

De Graaf, F. K. and P. Klaasen. 1986. Organization and expression of genes involved in biosynthesis of K99 fimriae. Molecular and General Genetics, 3: 508-514.

Evans, D. J. Jr., D. J. Evans, H. L. Du Pont, F. Orskov and I. Orskov. 1977. Patterns of loss enterotoxigenicity by *Escherichia coli* isolated from adults with diarrhea: suggestive evidence for an interrelationship with serotype. Infection and Immunity, 18: 105-111.

Gross, R. J., L. R. Ward, E. J. Threlfall, H. King and B. Rowe. 1982. Drug resistance among infantile enteropathogenic *Escherichia coli* strains isolated in the United Kingdom. British Medical Journal, 285: 472-473.

Hove, H., M. Tvede and P. B. Mortensen. 1996. Antibiotic associated diarrhea, *Clostridium difficle,* and short chain fatty acids. Scandinavian Journal of Gastroenterology.

Ipatenko, N. G. 1974. Infectious enterotoxaemia of camels. Veterinary Bulletin, 44: 1481.

Kado, C. I. and S. T. Liu. 1981. Rapid procedure for detection and isolation of large and small plasmids. Journal of Bacteriology, 145: 1365-1373.

Karmali, M. A. 1989. Infection by verocytotoxin-producing *Escherichia coli.* Clinical Microbiology Reviews, 2: 15-38.

Kunkel, S. L. and D. C. Robertson. 1979. Purification and chemical characterization of the heat-labile enterotoxin produced by enterotoxigenic *Escherichia coli*. Infection and Immunity. 25: 586-596.

Mooi, F. R., F. K. de Graaf and J. D. A. Van Embden. 1979. Cloning, mapping and expression of genetic determinant that encodes for K88ab antigen. Nucleic acid Research, 6: 849-865.

Moseley, S. L., G. Dougan, R. A. Schneider and H. W. Moon. 1986. Cloning of chromosomal DNA encoding the F41 and K88. Journal of Bacteriology, 167: 799-804.

Romboli, B. 1942. L. E. malattie di allevamento nei camelidi. Veterinaria, 85: 13-21.

Schifferliv, D. M., E. H. Beachey and R. K. Tayor. 1990. The 987P fimbrial gene cluster of enterotoxigenic *Escherichia coli* plasmid encoded. Journal of Bacteriology, 171: 149-156.

Tzipori, S. 1981. The aetiology and diagnosis of calf diarrhea. Veterinary Record, 108: 510-514.