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With 1 table in the text.

Fimbrial adhesins of *E. coli* Strains isolated from Diarrhoeic Calves in Khartoum State.

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ملخص البحث

عزلت 71 معزولةً من الأشريكية القولونية *E.coli* من عينات روث جمعت من عجول (أبقارحلوب) تعانى من الإسهال يتراوح عمرها ما بين يوم الي 21 يوماً لتحديد معزولات الإشريكيه القولونية المخملة. تم إجراء اختبار الخمل (FIMBREX; CVL .Weybridge, U.K.). على الـ71 معزولةً وجدت 30 (42%) منها موجبه لهذا الاختبار وتمتلك احد المستضدات الخملية F41 (18 معزولة)، K99 (6 معزولات)، K88 (6 معزولات) ولكنها لا تمتلك المستضد الخملي 987P.

Summary

Seventy one *Eschericia coli* (*E.coli*) strains, isolated from diarrhoeic dairy cattle calves in Khartoum State, were tested for the presence of fimbrial adhesins using the Fimbrex kits (CVL .Weybridge, U.K.). Thirty strains (42 %) were found to possess one of the fimbrial antigens, F41 (18 strains), K99 (6 strains) and K88 (6 strains). None of the strains tested had a 987P antigen.

Introduction

Pathogenic *Eschericia coli* (*E. coli*) is associated with many disease conditions including enteric involvements in animals and humans (Sojka, 1971; Moon, 1974); entrotoxigenic *E.coli* (ETEC) strains are of particular concern as they cause neonatal diarrhoea in both man and animal (Tzipori, 1981). The ETEC strains cannot be separated from non-pathogenic ones on the basis of their biochemical characteristics as there is no biochemical test that distinguishes them from one another. Therefore, to determine the pathogenic potential of an isolate of *E.coli*, one or more of the pathogenicity tests should be carried out (DuPont *et al.*, 1971; Guerrant *et al.*, 1974; Sack, 1975; Giannella, 1976; Gyles, 1994).

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Fimbrial adhesins which confer colonization ability to *E. coli* strains are convenient targets for the detection of enterotoxigenic strains. Hence, virulence attributes of ETEC include the adhesion of their pili or fimbriae to intestinal villous epithelial cells to prevent peristaltic elimination by the gut.

This study was carried out to identify the common fimbrial antigens of *E. coli* strains isolated from diarrhoeic diary cattle calves.

Materials and Methods

Samples were collected from farms in different locations in Khartoum state mainly from Soba, Kuku and Shambat, Gezira, Kennana and Atbara during the period 1998 – 2002. Three to five ml of watery faeces was collected from each diarrhoeic calf, 1-21 days old.

Isolation of microorganisms:

Samples were cultured within 2-3 hrs after collection. For primary isolation of the bacteria, a loopful of the specimen was taken aseptically from each sample and streaked onto plates of Blood Agar and MacConkey Agar to give well isolated colonies. Inoculated plates were aerobically incubated at 37ºC for 24 hrs.

Cultures on Blood Agar were examined for haemolysis and those on MacConkey Agar were observed for lactose fermentation. Subculturing was made on Nutrient Agar to obtain pure cultures which were further identified by applying primary and secondary biochemical tests according to Barrow and Feltham (1993).

Detection of fimbrial antigens:

FIMBREX (Central Veterinary Laboratory, Weybridge, U.K) are commercially available kits for *in vitro* detection of fimbrial adhesins of ETEC. It is a monoclonal antibody-based agglutination test (Morris and Sojka, 1985; Thorns *et al.,* 1987; Thorns and Roeder, 1988). Four kits were used; each kit was specific for one of the four fimbrial adhesins (K88, K99, F41 or 987P). The tests were performed by mixing equal volumes of test latex and suspensions of organisms on disposable white plastic-coated card, rocking gently for up to two minutes and observing any macroscopic agglutination. Auto-agglutination of test suspensions was checked by replacing the test latex with the control latex.

Results

Seventy one *E. coli* strains were isolated and tested in this study (Table 1). Thirty out of the seventy-one strains tested (42%) were found to possess one of the fimbrial antigens F41, K88 or K99 each and none of them possessed 987P antigen.

Table 1: Fimbrial adhesins possessed by *E. coli* strains isolated from diarrhoeic dairy cattle calves from different localities in the Sudan.

Localities	No. isolates tested	No. isolates possessing fimbrial antigens				No. negative
		K88	K99	K41	987p	
Dept. Path. (CVRL, Khartoum)	9	2	2	1	0	4
Hillat kuku (Khartoum North)	15	1	0	4	0	10
Fact. Vet. Med. Clinic, U. of K.	13	2	0	1	0	10
Arab Dairy Farm (ELBageir, Gezira)	6	0	2	1	0	3
Um Haraz (Gezira)	5	0	0	5	0	0
Butri (Gezira)	6	0	0	2	0	4
Masoudia (Gezira	5	0	0	1	0	4
Kennana area (White Nile)	9	0	2	2	0	5
Atbara Vet. Res. Lab. (Rivr Nile State)	3	1	0	1	0	1
Total	**71**	**6**	**6**	**18**	**0**	**41**

Discussion

Calf diarrhoea is most common in newborn animals, 2-10 days old; it may occur as early as 12-18 hours after birth and occasionally in calves of up to 3 weeks of age (Radostits and Acres, 1980). In the Sudan, recognition of fimbrial adhesins of *E. coli* isolates from cattle calves was not done before. However, Salih (1993) identified fimbrial adhesins in *E. coli* strains obtained from 66% of diarrhoeic camel calves. In our study *E. coli* was primarily isolated in pure culture from 80% of diarrhoeal faecal samples; a finding which may indicate the extent of *E. coli* involvement and its important role in the causation of diarrhoea. Comparable finding was also reported by Salih (1993) in camel calves.

The most significant adhesins of *E. coli* strains that are pathogenic to domestic animals and were looked for in this study, are K88(F4), K99(F5,), 987P (F6) and F41(Quinn *et al.*, 2001; Gyles, 1994). In the present study three adhesins, of which F41 was the commonest (60%) followed by K88(20%) and K99 (20%), were encountered using the FIMBREX kits. This method for adhesive gene determination was used for the first time in the Sudan. Salih *et al*. (1997) have recognized *E. coli* F41 and K88 strains, which were isolated from diarrhoeic camel calves, by DNA probing. Tzipori (1985) has reported the occurrence of K99 *E. coli* in 30-40% of diarrhoea in cattle and sheep. The K99 and F41 adhesins occur in calves and K99 occurs in lambs (Quinn *et al.*, 2001). The relative frequency of each one of the four adhesins differs between localities and seasons (Kaditerwakku and Harbour, 1990).

A complication for fimbrial identification arises from the fact that an isolate may produce a sub-clone, which may or may not produce a fimbrial antigen, whereas another one may produce multiple fimbriae of different types (Thorns *et al.*, 1989).

The importance of the adherence phenomenon in pathogenesis was demonstrated and emphasized by Beachey (1981). This property is essential in entrotoxigenic colibacillosis of calves, where the adhesin K99 is involved (Orskov *et al.*, 1975).

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References

Barrow, G. I. and Feltham, R. K. A. (1993). Cowan and Steel's Manual for the identification of medical bacteria, 3rd ed, Cambridge University Press, Cambridge, UK. Pp.

Beachey, E. H. (1981). *J. Infect. Dis.*, **143:** 325-345.

DuPont, J. L.; Formal, S. B.; Hotnick, R. B.; Snyder, M. J.; Libonati, J. P.; Sheahan, D. G.; Labrec, E. H. and Kales, J. P. (1971). *New Engl. J. Med.*, **285:** 1-9.

Giannella, R. A. (1976). *Infect. Immun.*, **14:** 95-99.

Guerrant, R. L.; Brunton, L. L.; Schnaitman, T. C.; Rebhun, I. and Gilman, A. G. (1974). *Infect. Immun.*, **10:** 320-327.

Gyles, C. L. (1994). *Escherichia coli* in domestic animals and humans. CAB International, Wallingford, England. Pp. 419-426.

Koditerwakku, S. N. and Harbour, D. A. (1990). *Vet. Rec.*, **126:** 547-549.

Moon, H. W. (1974). Pathogenesis of enteric diseases caused by *E.coli*. In: Advances in Veterinary Science and Comparative Medicine. Vol**. 18,** Academic press Toronto, Pp**.** 179- 211.

Morris, J. A. and Sojka, W. J. (1985). The Virulence of *E. coli*. In: M. Sussman (ed.). *Escherichia coli* as a pathogen in animals. Special publication of the Society of General Microbiology. Academic Press, London, U.K. No. **13:** 47-77.

Orskov, I.; Orskov, F.; Smith, H. W. and Sojka, W. J. (1975). *Acta Pathol. Microbiol. Scand.*, **Sect. B**. **83:** 31-36.

Quinn, P. J.; Markey, B. K.; Carter, M. E.; Donnelly, W. J. C. and Leonard, F. C. (2001). Veterinary Microbiology and Microbial Diseases. Ed. Dublin. Pp.

Radostits, O. M. and Acres, S. D. (1980). *Canad.Vet. J.*, **21:** 243-249.

Sack, R. B. (1975). *Ann. Rev. Microbiol*., **29:** 333-351.

Salih, O. S. M. (1993). Aetiological and epidemiological factors associated with camel-calf diarrhoea. M. V. Sc. Thesis, Facuty o Veterinary Science, University of Khartoum.

Salih, O. S. M.; Shigidi, M. T.; Mohamed, H. O. S.; McDough, P. and Chang, Y. F. (1997). Camel Research Newsletter No. **13**. pp. 132-137. The Arab Centre for the Studies of Arid Zones and Dry lands (ACSAD) P.O. Box 2440 Damascus, Syria.

Sojka, W. J. (1971). *Vet. Bull*., **41:** 309-322.

Thorns, C. J. and Roeder, P. L. (1989). *Res. Vet. Sci*., **44:** 394-5.

Thorns, C. J.; Boarer, C. D. A. and Morris, J.A. (1987). *Res. Vet. Sci*., **43:** 233-238.

Thorns, C. J.; Sojka, M. G. and Roederr, P. L. (1989). *Vet. Rec*., **125:** 91-92.

Tzipori, S. (1981). *Vet. Rec.*, **108:** 510-514.

Tzipori, S. (1985). The relative importance of enteric pathogen affecting neonates of domestic animals. In: C. E. Cornelius and C. F. Simpson (eds.) Advances in Veterinary Science and Comparative Medicine. Vol. **29.** Academic Press. Toronto. Pp. 103-206.