Indian Vet. J. 74, November, 1997 :915 - 918

CHARACTERIZATION OF CLOSTRIDIUM PERFRINGENS ISOLATES FROM GOATS *

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Clostridium perfringens is normally present in the intestines of healthy animals. They produce a variety of toxic and enzymic substances which have been studied in detail because of their importance in relation to the identification of strains and the pathogenesis of disease. Disease occurs when these intestinal organisms begin to multiply unusually rapidly and produce toxins. Among the various diseases caused by *C. perfringens*, enterotoxaemia is one of the most important and highly fatal diseases of sheep and goats throughout the world. The present work was undertaken to characterize strains of *C. perfringens* isolated from goats.

Materials and Methods

Collection of samples : The materials were collected from slaughtered goats suffering from watery to severe diarrhoea and dead goats showing typical post-mortem lesions of enterotoxaemia. Bacteriological samples were collected from duodenum and caecum with the help of sterile swabs.

Isolation and identification of C. perfringens : Primary isolation was made on 5% sheep blood agar plates to which 400 ug of D-cycloserine (Sigma Chemical Company, St. Louis, USA) per ml of melted agar was added. Plates were incubated anaerobically in a Gaspak system (BBL, Cockeysville, USA) for 24 hours at 37°C. One colony, showing a typical double haemolysis zone, from each plate was subcultured on a sheep blood agar plate without cycloserine. Passages were repeated on cycloserine-free 5% blood agar plates until the culture was considered pure. The isolates were identified according to the procedure described by Hauschild *et al.* (1979). Eleven randomly selected isolates were finally identified with the help of the Rapid ID 32 A system (API system, Biomerious, France) at the Laboratory of Veterinary Bacteriology, University of Liege, Liege, Belgium, Typing was done by DNA-DNA hybridization.

Gene probe and DNA-DNA hybridization.: The toxin type of the 11 isolates was obtained by mouse lethality assays (Sterne and Batty, 1975). Derivation of the 13 DNA probes used to classify isolates of *C. perfringers* is shown in Table-1. Eleven DNA probes derived from multicopy recombinant plasmids carrying the searched genes (Daube *et al.*, 1992; 1994a; Devriese *et al.*, 1993). Plasmid DNA was isolated by ultracentrifugation in cesium gradients as described by Sambrook *et al.* (1989). The other two gene probes were obtained by polymerase chain reaction

* Part of the Ph.D., thesis of the first author submitted to the Assam Agricultural University.

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(Daube *et al.*, 1994b). DNA colony hybridizations were performed as previsouly described (Daube *et al*, 1994a). Nineteen *C. perfringens* strains were used as controls in hybridization.

Results and Discussion

A total of 35 isolates of *C. perfringens* was isolated from 35 goats out of 200. All the isolates were confirmed with the available methods. The biochemical characterstics were in accordancd with those of Hauschild *et al.* (1979). Eleven of the 35 isolates which were obtained from 5 clinical cases of suspected enterotoxaemia and 6 diarrhoeic slaughtered goats, were further characterised. They were typed as *C. perfringens* type A on the basis of DNA-DNA hybridization.

Recovery of *C. perfringens* type A from healthy slaughtered goats was reported by Benaouda (1985). The recovery of *C. perfringens* type A from enterotoxaemic and diarrhoeic slaughtered goats in the present investigation was in accordance with the findings of Urban *et al.* (1975) and Volkova and Dzhamgyrchieva (1976). The clostridial organisms can produce disease under certain conditions such as sudden change in diet, over eating etc. The earlier reports showed that the cause of enterotoxaemia was either *C. perfringens* type D (Shanks, 1949; Wanasinghe, 1973) or type A (Volkova and Dzhamgyrchieva, loc.cit). Moreover, both the types were recovered from faeces of enterotoxaemic goats (Urban *et al.*, loc.cit). The epsilon-toxin of type D and alpha-toxin of type A were thought to be responsible for

Table 1-Derivation of the 13 DNA probes used to classify isoaltes of *Clostridium perfringens*

Probe	Origin	Endonuclease*	Size (base Pairs)	Reference
α	pTOX5†	*Bam*H1 and *Hin*dIII	950	Daube *et al.*, 1994a
β	PCR fragment	NA	1,025	Daube *et al.*, 1994b
ε	pGDS7†	*Hin*dIII	505	Daube *et al.*, 1994a
ta	PCR fragment	NA	297	Daube *et al.*, 1994b
Enterotoxin	pLWL10†	KonI and HindIII	504	Daube *et al.*, 1994a
O	pRT1B†	*Aee*l	736	Daube *et al.*, 1994a
μ	pNAG-S3†	*Hin*dIII	1,300	Daube *et al.*, 1994a
Sialidase	unnamed†	*Kpa*I and *Hin*dIII	1,400	Daube *et al.*, 1994a
TetP	pJiR39†	*Sph*I and *Eco*RI	800	Daube *et al.*, 1992
CatP	pJIR62†	*Hinf*I and *Eco*RI	631	Daube *et al.*, 1992
CatQ	pJIR260†	*Dra*I and *Pst*I	350	Daube *et al.*, 1992
ErmBP	pJIR233†	*Eco*RI and *Hin*dIII	500	Daube *et al.*, 1992
ERmQ	pJiR2422†	*Eco*RI and *Hin*dIII	380	Devriese *et al.*, 1993

* Used to derive the gene probe fragment from the plasmid
† Plasmid
PCR = polymerase chain reaction; NA=not applicable

Table-2 : DNA-DNA hybridization of 11 *C. perfringens* strains.

Isolate No.	Gene probes												
	1	2	3	4	5	6	7	8	9	10	11	12	13
61	+	-	-	-	-	+	+	+	-	-	-	-	+
62	+	-	-	-	-	+	+	-	-	-	-	-	+
63	+	-	-	-	-	+	+	-	-	-	-	-	+
64	+	-	-	-	-	+	+	-	-	-	-	-	+
65	+	-	-	-	-	+	+	-	-	-	-	-	+
66	+	-	-	-	-	-	+	-	-	-	-	-	+
67	+	-	-	-	-	+	+	-	-	-	-	-	+
68	+	-	-	-	-	+	+	-	-	-	-	-	+
69	+	-	-	-	-	+	+	-	-	-	-	-	+
610	+	-	-	-	-	+	+	-	-	-	-	-	+
611	+	-	-	-	-	+	+	-	-	-	-	-	+

*1= alpha-toxin gene;
2 = beta-toxin gene;
3 = epsilon -toxin gene;
4 = iota-toxin gene;
5 = enterotoxin gene;
6 = theta-toxine gene;
7 = sialidase gene'
8 = tetraciline resistance factor gene;
9 &10 = Chloramphenicol resistance probe:
11 & 12 = macrolide resistance probe;
13 = mu-toxin gene and

enterotoxaemia in goats. However, to draw a useful conclusion, further studies of this nature would have to be carried out.

The results of DNA-DNA hybridization with *C. perfringens* gene probes indicated that all the representative isolates possessed homologous genes with the alpha-toxin, mu-toxin and sialidase gene. Ecept one, all the isolates hybridized with the theta-toxin gene probe. Sialidase is an enzyme which produces necrotising effect on the intestinal mucosal membrane and the process may be further facilitated by the presence of the mu-toxin by its hyaluronidase activity.

One isolate hybridized with the tetracycline-resistance factor (tet-p) gene probes (Table-2). This isolate was found resistant to tetracycline in the *in-vitro* disc-diffusion test carried out on Wilkins Chalgren anaerobic agar.

Summary

Clostridium perfringens was isolated from 35 of 195 diarrhoeic slaughtered goats and 5 enterotoxaemic goats. All the isolates showed typical biochemical reaction and double zone of haemolysis in 5% sheep blood agar plates. Eleven representative isolates of *C. perfringens* hybridized with the alpha-toxin, mu-toxin and sialidase gene probes upon DNA-DNA hybridization with 13 *C. perfringens* gene probes. One isolate hybridized with the tet P gene probe. All but one strain hybridized with theta gene probe.

Acknowledgement

The authors are grateful to Dr. L.A. Devriese, Laboratory of Veterinary Bacteriology, University of Ghent, Ghent, Belgium for kindly providing the Gaspak system free of cost.

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