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ENTEROTOXIN PRODUCTION AT 4, 22, AND 37°C BY YERSINIA ENTEROCOLITICA AND YERSINIA ENTEROCOLITICA-LIKE BACTERIA ISOLATED FROM PORCINE TONSILS AND PORK PRODUCTS

By

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NESBAKKEN, T.: *Enterotoxin production at 4, 22, and 37°C by Yersinia enterocolitica and Yersinia enterocolitica-like bacteria isolated from porcine tonsils and retail pork products.* Acta vet. scand. 1985, *26*, 13—20. — Altogether, 71 strains of Yersinia enterocolitica and Yersinia enterocolitica-like bacteria from porcine tonsils and pork products were examined for their ability to produce enterotoxin using the infant mouse assay. Of these, 37 strains (52.1 %) produced enterotoxin at 22°C, 3 were positive at 4 and 22°C, and 1 was enterotoxigenic at 22 and 37°C. No strain was positive at all 3 temperatures. The highest prevalence of enterotoxin production at 22°C was detected in serotype O:11 (80.0 %), followed by O:3/biotype 4 (74.2 %), and O:12 (66.7 %). Enterotoxin production at 4°C was recorded in 2 (15.4 %) of the Yersinia kristensenii strains (O:11, O:12) and 1 of the Yersinia enterocolitica strains (O:3) examined. One Yersinia kristensenii strain (O:11) was enterotoxigenic at 37°C. The results indicate that enterotoxin production is a common feature of yersiniae isolated from porcine tonsils and pork products in Norway and may represent a possible source of food borne intoxication.

Yersiniae; enterotoxigenicity; serotype; biotype; food intoxication.

Many human and environmental isolates of Yersinia enterocolitica and Yersinia enterocolitica-like bacteria produce a heat-stable enterotoxin in vitro at 20 to 30°C (*Pai et al.* 1978, *Kapperud* 1980).

The highest prevalence of enterotoxin production has been reported among human clinical isolates (*Pai et al.* 1978, *Boyce et al.* 1979, *Kapperud* 1980, *Kapperud et al.* 1980). However, none of the clinical isolates has so far been shown to produce entero-

toxin at human body temperature (*Kapperud* 1982). On the other hand, enterotoxin production at 37°C as well as at 4 and 22°C seems to be quite common in Yersinia kristensenii (*Kapperud & Langeland* 1981, *Kapperud* 1982).

It has been suggested that pigs and food products of porcine origin are the most important sources of human Yersinia enterocolitica infections (*Mollaret et al.* 1979, *Hurvell* 1981). These bacteria are common among Norwegian slaughter pigs and in retail pork products (*Nesbakken & Kapperud, Nesbakken et al.* in press). However, no information on enterotoxin production by porcine Yersinia isolates in this country has been previously published. This investigation was therefore carried out to obtain relevant data.

MATERIAL AND METHODS

Bacterial strains

Two different groups of strains were selected: (i) 53 strains isolated from porcine tonsils (*Nesbakken & Kapperud* in press) and (ii) 18 strains isolated from retail pork products (minced pork, forcemeat, pork chops) (*Nesbakken et al.* in prees). The strains were biotyped and serotyped according to the methods and criteria of *Wauters* (1970, 1981) as described by *Nesbakken & Kapperud* (in press).

Preparation of sterile culture filtrates

Strains were inoculated into 5 ml tryptic soy broth (TSB) (Difco laboratories Inc., Detroit, Michigan, USA) with 0.6 % yeast extract (Difco). Broths were incubated in a roller drum (60 rev./min) for 48 h at 22 and 37°C, and for 1 week at 4°C, as described by *Kapperud* (1982). The cultures were subsequently centrifuged, and the supernatants filtered through Millipore filters (0.45 μm) under sterile conditions.

Enterotoxin assay

Enterotoxin activity was tested using the infant mouse assay (*Dean et al.* 1972). Sterile culture filtrates (0.1 ml) were administered by gastric tube into the stomachs of three 2—5 day — old mice as described by *Okamoto et al.* (1982). After being kept at room temperature for 4 h, the mice were killed, and the ratio of intestine weight to remaining body weight was deter-

mined. Ratios of $\geq$ 0.083 were accepted as positive (*Sack et al.* 1975). Strains which produced enterotoxin at 22°C were further tested at 4 and 37°C except strains belonging to biotype 4, serotype O:3 (only 4°C). The prevalence of enterotoxigenity varied with the source of isolation and with serotype affiliation.

RESULTS

Of 71 isolates of Yersinia enterocolitica and Yersinia enterocolitica-like bacteria, 37 (52.1 %) produced enterotoxin at 22°C

Table 1. Relationship between enterotoxin production and biotypes/serotypes of Y. enterocolitica and Y. enterocolitica-like bacteria isolated from porcine tonsils and pork products.

Species/Serotype	Strains isolated from porcine tonsils				Strains isolated from pork products			
	No. tested	No. positive at (°C) 22	4 22	37 22	No. tested	No. positive at (°C) 22	4 22	37 22
Y. enterocolitica biotype 4								
O:3	34	25	1	—*	1	1	0	—
Y. enterocolitica biotype 2								
O:5, 27	1	0	—	—				
Y. enterocolitica biotype 1								
O:5	2	0	—	—	3	1	0	0
O:6	2	0	—	—	1	0	—	—
O:7, 8	3	1	0	0				
O:10	1	0	—	—	1	0	—	—
O:25					2	1	0	0
O:41					1	1	0	0
NAG**	1	0	—	—				
Y. kristensenii								
O:3					1	1	0	0
O:5	1	0	—	—				
O:11	4	3	0	1	1	1	1	0
O:12					3	2	1	0
NAG	3	0	—	—				
Y. intermedia								
O:4, 33	1	0	—	—				
O:17					2	0	—	—
O:21, 46					1	0	—	—
NAG					1	0	—	—
Total	53	29	1	1	18	8	2	0

* Not tested at 4 or 37°C.

** NAG; not agglutinable with available antisera.

Table 2. Production of heat-stable enterotoxin by different Yersinia spp. isolated from porcine tonsils and pork products.

Species	Total no. of strains tested	No. (%) of strains enterotoxigenic at (°C)		
		22	4 22	37 22
Y. enterocolitica biotype 4 (O:3)	35	26 (74.2)	1 (2.9)	—*
Y. enterocolitica biotype 2 (O:5, 27)	1	0	—	—
Y. enterocolitica biotype 1	17	4 (23.5)	0	0
Y. kristensenii	13	7 (53.8)	2 (15.4)	1 (7.7)
Y. intermedia	5	0	—	—
Total	71	37 (52.1)	3 (4.2)	1 (1.4)

* Not tested at 4 or 37°C.

as indicated by the infant mouse assay. Of these 37 strains, 3 were also positive at 4°C, and 1 was enterotoxigenic at 37°C. No strain was positive at all 3 temperatures (Tables 1 and 2).

Serotypes

Altogether, enterotoxin production was recorded for 8 (61.5 %) of the 13 serotypes examined (Table 1). The highest prevalence of enterotoxigenic strains at 22°C was seen in serotype O:11 (80.0 %), followed by O:3/biotype 4 (74.2 %), and O:12 (66.7 %). Enterotoxin production at 4 and 22°C was recorded for 2 (15.4 %) of the Yersinia kristensenii strains (O:11, O:12) and 1 of the Yersinia enterocolitica strains (O:) examined. One Yersinia kristensenii strain (O:11) was enterotoxigenic at 37 and 22°C.

Source of isolation

Enterotoxin production at 22°C was indicated in 29 (54.7 %) of 53 strains from porcine tonsils. One of these strains was enterotoxigenic at 4°C and another at 37°C. Eight (44.4 %) out of 18 strains isolated from porcine products evoked a positive reaction at 22°C, and 2 of these were also positive at 4°C (Table 1). Nevertheless, there was no significant difference ($P > 0.05$) in the prevalence of enterotoxin production at 22°C among strains

isolated from these 2 sources ($\chi^2 = 0.57$). However, these groups were not strictly comparable since the spectrum of serotypes isolated was substantially different.

DISCUSSION

Strains belonging to serotype O:3/biotype 4 are by far the most frequent causal agents of human Yersinia enterocolitica enteritis in Europe (*Mollaret et al.* 1979, *Hurvell* 1981). In Norway, more than 99 % of recorded cases are due to this variant (*J. Lassen*, pers. comm. 1984). This bio-serotype is a common inhabitant of the intestinal tract and oral cavity of pigs in this part of the world (*Mollaret et al.* 1979, *Wauters* 1979, *Christensen* 1980, *Hurvell* 1981, *Nesbakken & Kapperud* in press).

The present study indicates that almost three-quarters (74.2 %) of the O:3/biotype 4 strains isolated from porcine tonsils and food products of porcine origin in Norway (Table 2) are able to produce enterotoxin at 22°C. In comparison, enterotoxin production was registered in 20 (91.0 %) of 22 strains belonging to biotype 4, serotype O:3 isolated from human patients in this country (*Kapperud* 1980). In a study of porcine O:3 isolates from Sweden, only 38 % of the strains were enterotoxigenic, whereas 83 % of the human O:3 isolates were positive (*Olsson et al.* 1980). *Schiemann* (1980) reported that 11 of 12 O:3 isolates from pork products in Canada were enterotoxigenic.

Enteropathogenic strains of Yersinia enterocolitica harbour a particular species of plasmid DNA which is essential for the expression of virulence (*Gemski et al.* 1980, *Portnoy et al.* 1981, *Vesikari et al.* 1981). Presence of this plasmid is correlated with the capability of spontanous autoagglutination (*Vesikari et al.* 1981). Accordingly, an autoagglutination assay (*Laird & Cavanaugh* 1980) has been described which provides a rapid and reliable presumptive indication of the virulence of Yersinia enterocolitica isolates. The autoagglutination characteristics of the strains included in the present study will be reported elsewhere (*Nesbakken & Kapperud, Nesbakken et al.* in press). Seven out of 21 (33.3 %) of the presumptive virulent O:3/biotype 4 strains lacked the ability to produce enterotoxin. Furthermore, 8 (30.8 %) of the enterotoxigenic strains were avirulent as judged by the autoagglutination assay. These results strongly indicate that enterotoxin production is neither necessary nor sufficient for

the expression of virulence in Yersinia enterocolitica. This conclusion is in accord with the results of other investigators (*Vesikari et al.* 1981, *Schiemann & Devenish* 1982). This is further supported by the fact that enterotoxin production at human body temperature is only detected in Yersinia kristensenii, a species with uncertain clinical importance. However, although the pathogenic significance of the heat-stable enterotoxin si dubious, the possibility still remains that enterotoxigenic strains may provoke food borne intoxication involving preformed enterotoxins (*Kapperud & Langeland* 1981). This assumption is based on observation that this toxin is able to resist gastric acid as well as the temperatures used in food processing and storage (*Boyce et al.* 1979). Enterotoxin production at 4°C which is a common storage temperature for perishable foods, has been found to be prevalent in Yersinia kristensenii (*Kapperud* 1982). In the present study enterotoxin production at 4°C was recorded for 2 (15.4 %) of the Yersinia kristensenii strains.

In conclusion, the present study demonstrates that enterotoxin production is common among porcine isolates of Yersinia in Norway. The question whether such strains are capable of causing food borne intoxication remains unsettled.

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SAMMENDRAG

Enterotoksinproduksjon ved 4, 22 og 37°C hos Yersinia enterocolitica og Yersinia enterocolitica-lignende bakterier isolert fra tonsiller hos gris og matvarer med svinekjøtt som vesentlig bestanddel.

I alt ble 71 stammer av Yersinia enterocolitica og Yersinia enterocolitica-lignende bakterier undersøkt for enterotoksinproduksjon in vitro (infant mouse assay). Trettisju (52,1 %) av stammene produserte enterotoksin ved 22°C, og 3 av dem ved 4 og 22°C, mens 1 stamme var enterotoksinproduserende ved 22 og 37°C. Høyeste frekvens av enterotoksinproduserende stammer ved 22°C ble funnet innen serotype O:11 (80,0 %), etterfulgt av O:3/biotype 4 (74,2 %) og O:12 (66,7 %). Enterotoksinproduksjon ved 4°C ble funnet hos 2 av Yersinia kristensenii — stammene (O:11, O:12) og 1 av Yersinia enterocolitica — stammene (O:3) som ble undersøkt. En Yersinia kristensenii — stamme (O:11) var positiv ved 37°C. Resultatene viser at enterotoksinproduksjon er svært vanlig hos Yersinia-bakterier isolert fra svinetonsiller og matvarer med svinekjøtt som vesentlig bestanddel i Norge.

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