ENUMERATION OF YERSINIA SP. FROM THE ORAL CAVITY OF FRESHLY SLAUGHTERED SPANISH PIGS

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INTRODUCTION

Sporadic human infections with Yersinia enterocolitica are quite common in Europe and they have been also reported in Spain. It has been suggested that pigs and food products of porcine origin constitute the major reservoir of human infections with Y. enterocolitica and many studies have shown that pigs may be asymptomatic carriers of strains which belong to the same serotypes, biotypes and phage types as those associated with human disease (Mollaret et al. 1979; Christensen, 1980; Nesbakken and Kapperud, 1985; Nesbakken, 1988).

Further evidence that pigs are the reservoir of human infection with Y. enterocolitica is supported by the fact that it has not been possible to distinguish between human and porcine strains, neither by biochemical, serological or phage-typing procedures (Wauters, 1979) nor by plasmid DNA screening methods (Nesbakken et al. 1987; Shiozawa et al. 1987). There is also a strong correlation between the serogroups of Y. enterocolitica isolated from humans and pigs in the same geographical area (Pedersen, 1979). In Belgium, a case-control study has shown that Y. enterocolitica infection was strongly associated with eating raw pork (Tauxe et al. 1987), but on the other hand, not a single case of human yersiniosis has been reported to date in which pigs or pork products were clearly identified as the vehicle. This work reports on the isolation of Yersinia sp. from the oral cavity of freshly slaughtered Spanish pigs.

MATERIALS AND METHODS

Collection of samples

Tongues from 246 freshly slaughtered pigs from 4 different lots and collected during February to June 1987, were examined for the presence of yersiniae. Samples were from three slaughterhouses located in and around Madrid. All pigs were processed to the point just subsequent to evisceration, when tongues were rubbed thoroughly with a sterile cotton wool swab.

Isolation procedure

The swabs were subsequently placed in tubes containing 5 ml of a low selectivity medium, consisting of phosphate buffered saline (PBS, 1/15 M, pH 7.6), supplemented with 1% sorbitol and 0.15% bile salts (Mehlman et al. 1978). Prior to incubation, the PSB tubes were sealed with parafilm to minimize available oxygen and a three-week cold enrichment was accomplished by further incubation of the PSB cultures at 4 ºC. After the enrichment period, two loopfuls were finally palted out onto Cefsulodin-Irgasan-Novobiocin agar (CIN agar), commercially obtained as Yersinia Selective Agar Base and Supplement from Oxoid.

Identification of Yersiniae

Colonies resembling Yersiniae sp. on CIN agar were subcultured for a preliminary biochemical screening in MacConkey agar and in the LAIA medium (lysine-arginine-iron agar), devised for the presuntive identification of Y. enterocolitica by Weagant (1983). Suspect isolates were subjected to additional biochemical and subcultural characterization. Altogether, each isolate was tested by a number of parameters such as lysine, arginine and ornithine decarboxilase, lysine and phenilalanyne desaminase, β-galactosidase, urease, oxidase, citrate (Simmon's), H_2S production, lecithinase activity, motility, nitrate reductase, indole production and acid production from xylose, glucose, lactose, rhamnose, saccharose, mannitol, melibiose, threalose and raffinose. The parameters listed above formed the basis for identification of Y. enterocolitica and related species according to establis-

hed criteria (Bercovier and Mollaret, 1984).

Biotyping and serotyping

Isolates identified as Y. enterocolitica were biotyped by the methods and criteria of Bercovier and Mollaret (1984). Serlogical typing was carried out at the Institute Pasteur, Paris, by courtesy of Dr. Mollaret.

RESULTS

Yersinia sp. were isolated from the tongues of 40 (16.2%) of 246 freshly slaughtered pigs (Table 1). Y. enterocolitica comprised 92.5% (n=37) of the total number, followed by Y. intermedia (n=1), Y. kristensenii (n=1) and Y. frederiksenii (n=1) with a 25% of the isolates. Three biotypes and seven different serotypes were recognized (Table 1). The most frequently encountered serotype was O:3 which comprised 70% of the total number, followed by O:7,18,13,19; O:18, 19 and O:5 which comprised, respectively, 7,5% of the serotypes and the O:52,53, 54; O:12,25 and O:14,16,19 comprising, respectively, 2.5% of the serotypes.

Y. enterocolitica O:3/biotype 4, the predominant human pathogen in Europe was isolated from 11.3% of the pigs examined. All of these lots were found to be infected with serotype O:3/biotype 4, and the carriage rate ranged from 4.7% to 21.6%.

CONCLUSIONS

Many surveys, have demonstrated the common ocurrence of Y. enterocolitica and related microbes in the intestinal tract and oral cavity of healthy slaughter pigs. Our present results demonstrate that Y. enterocolitica and related microbes are also common in the oral cavity of Spanish slaughter pigs. However, the relative difficulty in isolating yersiniae from these samples may suggest that the percentage of positive cultures is still understimated. Recently, Wauters et al. (1988) have reported that the plating of ITC enrich

TABLE I. Serological and biochemical characterization of 40 yersiniae from porcine tongues

Serotype	Nº of isolates	% of total
Y. enterocolitica biotype 4		
O:3	28	70
Y. enterocolitica biotype 1		
O:7,8,13,19	3	7.5
O:5	2	5.0
O:8,19	3	7.5
Y. enterocolitica biotype 3		
O:5	1	2.5
Y. intermedia		
O:52,53,54	1	2.5
Y. kristensenii		
O:12,25	1	2.5
Y. frederiksenii		
O:14,16,19	1	2.5

ments (modified Rappaport base, supplemented with Irgasan, ticarcillin and potassium chlorate) onto SS-deoxycholate calcium agar (modified SS-deoxycholate agar, containing 1% deoxycholate and 0.1% CaCl), gave overall better results than platting onto CIN agar for serogroup O:3.

Not all yersiniae are clinically significant (Mollaret et al. 1979); Van Noyen et al. 1981). Strains belonging to serotype O:3/biotype 4 constitute most of the human clinical isolates in Spain (Gurgi et al. 1988). This bio-serotype was recovered from 11.3% of the pigs examined, which is lower than the range reported from other European countries (Nesbakken and Kapperud, 1985). The isolation procedures strongly influence the kinds of Yersinia strains encountered, since no single method has been described that will perform equally well for recovery of all kind of Yersinia sp. (Nesbakken and Kapperud, 1985; Wauters et al. 1988).

These results confirm the presence of Yersinia sp. in the oral cavity of freshly slaughtered Spanish pigs and they also suggest that the porcine oral cavity may also represent a considerable source of contamination of the pigs carcasses and a major reservoir of potential human infection with Y. enterocolitica. Based onto the possible spread of Y. enterocolitica from the oral cavity to other organs, the carcasses and the floor and environment of the slaughterhouse, European investigators have already emphasized the need for changes to be introduced in slaughtering technology and meat inspection practices (Christensen, 1987; Nesbakken, 1988).

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