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COMPARISON OF SAMPLING AND ISOLATION PROCEDURES FOR RECOVERY OF YERSINIA ENTEROCOLITICA SEROTYPE O:3 FROM THE ORAL CAVITY OF SLAUGHTER PIGS

By

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NESBAKKEN, TRULS: *Comparison of sampling and isolation procedures for recovery of Yersinia enterocolitica serotype O:3 from the oral cavity of slaughter pigs.* Acta vet. scand. 1985, *26*, 127—135. — Yersinia enterocolitica serotype O:3/biotype 4 was isolated from the oral cavity of altogether 32 (68.1 %) of 47 freshly eviscerated slaughter pigs. Most efficient recovery was achieved by cultivation of tissue samples from both tongue and tonsils of the same individual. The isolation rate so obtained was significantly higher than that obtained by separate examination of either tonsil swabs or tongue swabs. However, the isolation frequency achieved by combined swabbing of the 2 sites was not significantly different. In general, tonsils were more productive for the recovery of O:3 strains than were tongues, and tissue samples yielded higher isolation rates than did swabs. Three-week cold enrichment in a low selective medium proved essential for optimal recovery. However, the highest number of isolates was obtained using a combination of methods, including direct plating and selective enrichment in a modified Rappaport broth in addition to cold enrichment.

Yersinia enterocolitica; serotype O:3; sampling procedures; isolation methods.

Many studies have demonstrated that pigs may be healthy carriers of Yersinia enterocolitica (*Mollaret et al.* 1979, *Hurvell* 1981). A significant proportion of the bacteria encountered belongs to the same serotypes as those responsible for human Y. enterocolitica enteritis (*Wauters* 1979, *Hurvell* 1981). In addition to being an intestinal commensal, this bacterial species also inhabits the oral cavity of pigs (*Mollaret et al.* 1979, *Christensen* 1980, *Hurvell* 1981). In fact, the isolation frequencies recorded

on examination of tongues, tonsils, or throat swabs have proved to be substantially higher than those obtained from faeces or intestinal contents (*Pedersen* 1979, *Wauters* 1979, *Schiemann* 1980). During commercial slaughtering and processing, bacteria from the oral cavity may easily contaminate the carcasses, a circumstance which represents a potential hazard to consumers. Moreover, porcine tongues are popular foods in many countries, especially as cold cuts. Although adequate cooking will kill Y. enterocolitica, unhygienic handling of raw tongues may cause widespread dissemination of the organism to knives, cutting boards etc., and other food may become cross contaminated from the raw product. The ability of Y. enterocolitica to multiply in properly refrigerated foods (*Hanna et al.* 1977, *Stern et al.* 1980), further increases the risk of clinical infection.

In a concurrent study (*Nesbakken & Kapperud* in press), Y. enterocolitica serotype O:3, the common human pathogen in Europe, was isolated from the tonsils of 31.7 % of freshly eviscerated slaughter pigs in Norway. Similar results have been reported from other countries (*Christensen* 1980, *Hurvell* 1981). Thus, Y. enterocolitica is a microbe which should be of major concern to food inspection services. In light of the importance that efficient sampling and isolation procedures have, the present study was undertaken to compare the relative efficacy of different sampling methods and isolation procedures for the recovery of Y. enterocolitica serotype O:3 from the oral cavity of pigs.

MATERIALS AND METHODS

Collection of samples

During May 1984, tongues and tonsils from a total of 47 freshly eviscerated, healthy slaughter pigs were examined for the presence of Y. enterocolitica. The samples originated from 3 different herds which were represented by 5, 20, and 22 individuals, respectively. All samples were collected from one federally inspected slaughterhouse in Oslo. When the pigs had been processed to the point just subsequent to evisceration, the tongue and both tonsils were removed aseptically and placed in sterile polystyrene bags. Samples were transported at ambient temperature to the laboratory where cultivation was initiated within 2 h of collection.

Isolation

Both swabs and tissue samples were examined in order to determine the kind of sample which was most conducive to the recovery of Y. enterocolitica.

S w a b s: The upper surface of the tongue and both tonsils of each animal investigated were swabbed thoroughly with sterile cotton wool swabs. Tongue and tonsils were sampled and examined separately. Swabs were plated out immediately on cefsulodin-irgasan-novobiocin (CIN) agar (nos. CM 653 and SR 109; Oxoid Ltd., Basingstoke, Hampshire, England), and subsequently placed in tubes containing 10 ml phosphate-buffered saline (pH 7.6) containing 1 % sorbitol and 0.15 % bile salts (Oxoid) (PSB).

T i s s u e s a m p l e s: Tongues were prepared for examination by cutting surface portions (epithelium, mucosa, and part of the muscularis) from the upper rear of the organ. Tonsils were processed intact. Ten grams of each specimen were homogenized with 90 ml PSB in a Colworth 400 stomacher (A. J. Seward, London, England). After incubation at room temperature (20—25°C) for 3 h, 0.1 ml was plated out onto CIN agar.

The subsequent enrichment procedure followed the same course, regardless of the kind of sample involved. After pre-enrichment at 4°C for 8 days, 0.1 ml of the PSB cultures was transferred to 10 ml of a modified Rappaport broth (MRB) (no. 15209; E. Merck, Darmstadt, Germany) containing 80.0 g $MgCl_2$ per liter. As devised by *Schiemann* (1982), carbenicillin was omitted from MRB to avoid inhibition of certain sensitive serotype O:3 strains. Selective enrichment in MRB proceeded for 4 days at room temperature, and 0.1 ml was then plated out on CIN agar. Three-week cold enrichment was accomplished by further incubation of the PSB cultures at 4°C for another 14 days, and 2 loopfuls were finally plated onto CIN agar. All plates were incubated at 28°C and read after 18—22 h.

Identification

Suspect colonies were subcultured on lactose bromothymol-blue agar for further morphological inspection. Cultures resembling Yersinia spp. were then subjected to a primary biochemical screening using the three-tube method described by *Lassen* (1975), followed by a more extensive biochemical and cultural

characterization as detailed elsewhere (*Nesbakken & Kapperud* in press). Y. enterocolitica was identified according to established criteria (*Bercovier et al.* 1980).

Biotyping and serotyping

Only strains belonging to Y. enterocolitica serotype O:3/biotype 4 were considered in this study. Isolates were biotyped by the methods and criteria of *Wauters* (1970). Serological screening was carried out by slide agglutination against an absorbed rabbit antiserum representing O-antigen factor 3 (*Wauters* 1981).

Virulence assay

All isolates of serotype O:3/biotype 4 were tested for spontaneous autoagglutination at 22 and 37°C as described by *Laird & Cavanaugh* (1980), except that the medium was changed to Eagle's basal medium containing 10 % foetal calf serum. The procedure followed has been detailed elsewhere (*Nesbakken & Kapperud* in press). This assay represents a rapid presumptive indication of the pathogenic potential of Yersinia isolates.

RESULTS

Isolation frequencies

Altogether, Y. enterocolitica serotype O:3/biotype 4 was isolated from the oral cavity of 32 (68.1 %) of 47 freshly eviscerated slaughter pigs. Isolates were obtained from 2 of the 3 herds represented in the study. The recovery rates recorded from each particular herd were (total no. of individuals in parentheses): 81.8 % ($n = 22$), 70.0 % ($n = 20$), and 0 % ($n = 5$).

Comparison of sampling procedures

Several different sampling procedures were compared for their relative efficacy to recover Y. enterocolitica from naturally contaminated pigs (Table 1). Best recovery was achieved by cultivation of tissue samples from both tongue and tonsils of the same individual. This approach detected all the 32 pigs which were positive in this work. The isolation rate so obtained was significantly higher than that obtained by separate examination of either tonsil swabs ($\chi^2 = 5.23$, $P < 0.025$) or tongue swabs ($\chi^2 = 4.35$, $P < 0.05$) alone. However, the isolation frequency found by swabbing both organs was not significantly different

Table 1. Comparison of sampling procedures for recovery of Y. enterocolitica O:3 from the oral cavity of pigs.

Sampling procedure	No. of positive samples[a]
Tongue swab	21
Tonsil swab	22
Tongue swab + tonsil swab	24
Tongue tissue	26
Tonsil tissue	28
Tongue tissue + tonsil tissue	32
All methods	32

[a] A total of 47 pigs were investigated.

($\chi^2 = 2.83$, $P > 0.05$). In general, tonsils were more productive for the recovery of O:3 strains than were tongues, and tissue samples yielded higher isolation rates than did swabs.

Comparison of isolation procedures

Table 2 shows the recovery achieved using different combinations of isolation procedures. Three-week cold enrichment (PSB) proved essential for optimal recovery. This time consuming enrichment, being the most efficient single method employed, should be included in any combination of isolation methods,

Table 2. Comparison of isolation procedures for recovery of Y. enterocolitica O:3 from the oral cavity of pigs.

Isolation procedure	No. of positive samples				All samples
	Tongue		Tonsils		
	Swab	Tissue	Swab	Tissue	
P[a]	15	10	12	17	22
PSB-MRB[b]	12	16	12	18	24
P + PSB-MRB[c]	20	17	15	23	29
PSB	18	19	17	25	31
P + PSB	20	24	20	27	31
PSB + PSB-MRB	19	25	21	26	32
All methods	21	26	22	28	32

[a] P; direct plating of swabs, or plating of homogenized tissue samples after storage at room temperature (20—25°C) for 3 h.

[b] PSB-MRB; pre-enrichment in PSB (8 days, 4°C) followed by selective enrichment in MRB (4 days, 20—25°C).

[c] PSB; cold enrichment in PSB (3 weeks, 4°C).

regardless of the sampling procedure adopted. Three-week cold enrichment recovered more O:3 strains than either direct plating (P) or the two-step PSB-MRB approach, which was based on pre-enrichment in PSB followed by selective enrichment in MRB. However, neither of these differences were statistically significant. As might be expected, the highest number of isolates was obtained when all methods were combined.

Virulence assay

Of the 32 pigs which harboured Y. enterocolitica serotype O:3, 20 yielded only autoagglutination-negative strains, 6 yielded only autoagglutination-positive (AA+) strains, whereas the remaining 6 pigs were found to harbour both variants. AA+ strains were recovered from the tonsils of 7 pigs, from the tongue of 1 pig, and from both organs of 4 pigs.

DISCUSSION

Several authors have reported the isolation of Y. enterocolitica serotype O:3/biotype 4 from the oral cavity of pigs (*Narucka & Westendoorp* 1977, *Pedersen* 1979, *Wauters* 1979, *Christensen* 1980, *Schiemann* 1980, *Doyle et al.* 1981, *Hurvell* 1981, *Weber & Knapp* 1981, *Nesbakken & Kapperud* in press). The recovery rates range from 6.5 to 56.0 %. One factor which may influence the recovery rate, is the kind of specimen selected for examination. In a comparative study conducted in Ontario, Canada, *Schiemann & Fleming* (1981) concluded that thoat swabs and tongues were more productive for the recovery of Y. enterocolitica serotype O:3 than were tonsils. The incidence of this serotype was found to vary from 20 % for tonsils to 50 % for throat swabs and 55 % for tongues. However, the number of samples of each kind was relatively low ($n = 20$), and it would appear that neither of the differences observed were statistically significant ($P > 0.05$). Furthermore, the authors were not certain that their samples represented the same herd of animals, although swabs and tonsils were collected on the same day. As mentioned by *Christensen* (1980) and by *Schiemann & Fleming* (1981), carriage rates vary significantly between herds of pigs. Consequently, it cannot be ascertained whether the differences observed are attributable to the kind of sample examined, or to variations within herds.

In order to circumvent this problem, all samples compared in the present study were collected from the same individuals. It was found that examination of homogenized tissue samples from both tongues and tonsils gave the highest isolation rates. This approach, however, is substantially more time consuming than swabbing, and depends on the availability of appropriate homogenization equipment. Moreover, this method has economical implications, since it involves removal and destruction of an edible part, i.e. the tongue. The present results indicate that swabbing of tongue and tonsils would be an adequate alternative approach, especially for the screening of large series of animals. If further simplification is required, cultivation of tissue samples from the tonsils would be the method of choice.

All recovery rates are of course relative to the isolation procedure employed. The present results provide further evidence to support the conclusion made in a previous study (*Nesbakken & Kapperud* in press), that 3-week cold enrichment in PSB is essential for the recovery of Y. enterocolitica serotype O:3 from the oral cavity of pigs. A more comprehensive discussion of this observation has been presented elsewhere (*Nesbakken et al.* in press). In any case, more than one isolation method is required if optimal recovery is to be achieved.

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SAMMENDRAG

En sammenligning av prøvetakings- og isolasjonsprosedyrer ved undersøkelse av Yersinia enterocolitica serotype O:3 i munnhulen hos slaktegriser.

I alt ble Y. enterocolitica serotype O:3/biotype 4 isolert fra tunger eller tonsiller fra 32 (68,1 %) av 47 nylig slaktede griser. Flest antall isolater fremkom etter dyrking av vevsmateriale fra både tunge og tonsiller hos samme individ. Denne prosedyren gav maksimal isolasjonsrate og var signifikant bedre enn svabermetoden nyttet på enten tunge eller tonsiller. Dersom det ble foretatt svabring av begge organer, var det imidlertid ingen signifikant forskjell. Tonsiller syntes å være en bedre kilde enn tunger når det gjelder O:3 stammer. Tre ukers kuldeoppformering var den beste enkeltmetoden for isolasjon av O:3 stammer. Flest isolater ble oppnådd ved kombinasjon av flere metoder, inkludert direkte utsæd og selektiv oppformering i modifisert Rappaport buljong i tillegg til kuldeoppformering.

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