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Characterization of *Yersinia enterocolitica* strains isolated from cattle, sheep and pigs in the United Kingdom

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SUMMARY

The properties of 48 cultures identified as *Yersinia enterocolitica* or *Y. enterocolitica*-like organisms, including *Y. frederiksenii*, *Y. intermedia* and *Y. kristensenii*, were examined. Of these, 39 were isolated from faeces of apparently healthy pigs, five from healthy cattle, one from an aborted bovine fetus, one from an aborted lamb and one from a lamb suffering from acute enteritis.

Most isolates from healthy animals were of *Y. enterocolitica* biotype 1 or *Y. intermedia* and belonged to O serogroups not usually associated with disease in man or animals. The isolates from abortion or enteritis cases were of *Y. enterocolitica* biotypes 3, 4 and 5 and belonged to the pathogenic serogroups 0:5b and 0:2a, 2b, 3. No organisms of serogroup 0:9 were found.

INTRODUCTION

Yersinia enterocolitica is now widely recognized as a cause of disease in man and occasionally other species (Mollaret & Lucas, 1965; Langford, 1972; Winblad, 1973; Bottone, 1977). The source of most infections is unknown but outbreaks have been attributed to the consumption of contaminated food or water (Lassen, 1972; Eden *et al.* 1977; Black *et al.* 1978). In some localities food and water have frequently been found to be contaminated with *Y. enterocolitica* (Lee, 1977; Saari & Jansen, 1979; Schiemann, 1979) although the source or manner of contamination has seldom been identified.

Domestic animals, and in particular pigs, have been widely suspected of acting as a source of pathogenic *Y. enterocolitica* and in several countries evidence for this has been obtained (Esseveld & Goudzwaard, 1973; Leistner *et al.* 1975; Wauters, 1979). In the United Kingdom human infections with these organisms have been identified with increasing frequency in recent years (Anon, 1980). Nevertheless, there is no published information on the prevalence of *Y. enterocolitica* in domestic animals in this country. The present study was undertaken to remedy this omission.

MATERIALS AND METHODS

Sources of Yersinia *strains*

Three strains were received from veterinary investigation centres. All had been isolated from animals with overt disease. *Y. enterocolitica* strain 474 was isolated

post-mortem from the intestinal contents of a lamb with acute enteritis. *Y. enterocolitica* strain 47/78 was isolated from the stomach contents of an aborted bovine fetus and strain S.62 was isolated from ovine abortion material. The other strains described were isolated at this laboratory from apparently healthy cattle or pigs sent for slaughter.

Isolation procedures

Samples of faeces or caecal contents were collected on alginate swabs and transported in 10 ml volumes of phosphate buffered saline (PBS; 0·15 M-NaCl; 0·01 M phosphate buffer, pH 7·6). Samples of solid tissues were homogenized in 10–20 volumes of PBS. Portions of each sample were plated out directly onto serum dextrose agar, MacConkey agar, *Salmonella–Shigella* agar and Y medium (Soltész, Schalén & Mårdh, 1980) and incubated aerobically at 22 °C for up to 5 days. The remaining portion of each sample in PBS was incubated at 4 °C for 21 days and then plated as above.

The sensitivity of these isolation procedures was assessed by adding 1·75 g of fresh bovine or porcine faeces to 1 ml of serial dilutions of *Y. enterocolitica* 0:9 strain 296/68 in nutrient broth and stirring with an alginate swab which was then broken off and left in the suspension. The suspensions were incubated at room temperature (18 °C) overnight and then cultured as decribed above. The ability of the four agar media to support the growth of 50 reference strains, representing nearly all of the recognized serogroups of *Y. enterocolitica* was also evaluated.

Culture identification

Colonies resembling those of *Y. enterocolitica* reference strains were examined by preparing Gram-stained smears. Gram negative bacilli with a cellular morphology consistent with *Yersinia* were sub-cultured onto nutrient agar and incubated aerobically overnight, for further testing. Organisms were provisionally identified as *Y. enterocolitica* or related bacteria if they were oxidase negative but produced catalase, urease and ornithine decarboxylase and were positive for acid but not H_2S on triple sugar iron agar slopes.

Confirmatory and supplementary tests

Generic identity was confirmed by disc electrophoresis of phenol-acetic acid-water extracts (Corbel, Brewer & Hendry, 1982). Determination of biotype and serogroup (Bercovier *et al.* 1980) and of phage type (Nicolle, Mollaret & Brault, 1976) was done by Professor H. H. Mollaret of the Insitut Pasteur, Paris, France. Determination of biotype and serogroup according to Winblad (1979) was carried out by Professor S. Winblad, Department of Clinical Bacteriology. Allmänna-Sjukhus, Malmö, Sweden.

Tests for pathogenicity

Five tests for pathogenicity were used:

(1) Ability to establish lethal infection in gerbils (*Meriones unguiculatus*) was determined by intra-peritoneal injection of doses of approximately 10^6 viable organisms prepared as described previously (Schiemann & Devenish, 1980; Corbel, 1981). A full necropsy and cultural examination was performed on all animals dying of acute infection and those surviving at 7 days after inoculation.

(2) *Examination for enteropathogenicity in mice* was performed as described by Schiemann, Devenish & Toma (1981). The cultures were administered in the drinking water at a concentration of *ca.* 10^{10} viable organisms per ml for 7 days.

(3) *The Serény test* for ability to invade guinea-pig conjunctiva was done as described by Schiemann & Devenish (1980).

(4) *The auto-agglutination test* for virulence was done according to Laird & Cavanaugh (1980).

(5) *Examination for growth inhibition* and V and W virulence antigen production on magnesium oxalate-trypticase soy agar was done as described by Schiemann & Devenish (1980).

RESULTS

Sensitivity of isolation procedure

All of the reference strains grew on Y medium. However, not all strains grew as well on this medium as on the non-selective media. Thus some strains of 0 serogroups, 3, 4, 32, 5, 8, 15 and 22 grew more slowly on Y medium and produced smaller colonies than on Oxoid nutrient agar. These strains would probably have not been isolated from small inocula plated on Y medium.

All strains grew well on one or more of the other media used and the concurrent use of these permitted the isolation of all of the *Y. enterocolitica* serogroups examined.

Tests with *Y. enterocolitica* 0:9 strain 296/68 showed that Y medium was much more effective than any of the other media for isolating the organism from grossly contaminated material. Thus, the minimum inoculum of yersinias required for isolation from bovine or porcine faeces by direct plating was $5{\cdot}7 \times 10^{11}$/g for serum dextrose agar or *Salmonella–Shigella* agar, $5{\cdot}7 \times 10^{10}$/g for MacConkey agar and $5{\cdot}7 \times 10^{4}$/g for Y medium. When cold enrichment was combined with plating on Y medium, the minimum inoculum of organisms detectable was reduced to 57/g of faeces.

These results were confirmed by the examination of slaughter samples. Of the 45 *Yersinia* strains isolated from bovine or porcine faeces or tissues, all except 6 grew on Y medium. Of the 6 strains (all from porcine samples), four were isolated on MacConkey agar and one each on serum dextrose and *Salmonella–Shigella* agar but none were detected on Y medium.

Isoation of Yersinia *strains*

The results of culturing material from apparently healthy cattle and pigs for *Yersinia* species are summarized in Table 1. The highest proportion of isolates was obtained from porcine faeces or caecal contents. A much lower isolation rate was obtained from the bovine material.

Identification of Yersinia *isolates*

The results are shown in Table 2.

Pigs

Most isolates from pigs belonged to *Y. enterocolitica* biotype 1. A single strain 1339E, was identified as biotype 2. Seven strains were identified as *Y. intermedia*, two as *Y. frederiksenii* and one as *Y. kristensenii*. One strain, 1506M, was

Table 1. *Isolation of* Y. enterocolitica *and related organisms from apparently healthy animals sent for slaughter*

	No. of animals sampled	No. of samples tested		No. of samples positive for *Yersinia* (%)	No. of strains isolated
Cattle	598	Faecal/caecal:	576	2 (0·35)	3
		Tissues:	226	3 (1·33)	3
Pigs	889	Faecal/caecal:	889	36 (4·1)	39
Total	1487		1619	41 (2·4)	45

biochemically atypical and could not be classified within the framework of the recognized species. It most closely resembled *Y. intermedia* but did not ferment inositol and gave a protein migration pattern typical of *Y. enterocolitica* on disk electrophoresis of its acid-phenol extract.

Twelve isolates were not identified beyond the species level but most had properties consistent with *Y. enterocolitica* biotype 1.

All of the porcine isolates belonged to the X_o or the X_z phage type. They covered a whole range of 0:serogroups but with 6, 7, 8, 10K and 27 as the most frequently represented. None of the common human pathogenic 0 serogroups 3, 5b, 8 or 9 were represented among the porcine strains.

Cattle

Five of the seven cultures obtained from cattle were identified as *Y. enterocolitica. pseudotuberculosis* strains described by Kapperud, Bergan & Lassen (1981) and was identified by Professor S. Winblad as *Y. pseudotuberculosis*. Strain 47/78, isolated produce indole. It fermented melibiose and raffinose but not sorbose or rhamnose. Strain 1172Y did not produce indole but hydrolyzed aesculin and fermented melibiose, raffinose and rhamnose. Strain 2417E presented considerable difficulty in identification. It was ornithine decarboxylase-positive but atypical of *Y. enterocolitica* in other respects. It resembled in some respects the atypical *Y. pseudotuberculosis* strains described by Kapperud, Bergan & Lassen (1981) and was identified by Professor S. Winblad as *Y. pseudotuberculosis*. Strain 47/78, isolated from bovine abortion material, was identified as *Y. enterocolitica* biotype 4.

Sheep

The two ovine cultures, 474 and S.62, were of types usually associated with disease. Thus strain 474 was identified as *Y. enterocolitica* biotype 5, 0 serogroup 2a, 2b, 3 and phage type XI, properties typical of strains associated with infection in hares in Europe. Strain S.62 was identified as *Y. enterocolitica* biotype 3, 0 serogroup 5b and phage type X_z, a pattern commonly associated with human infection in Canada but rare in Europe.

Pathogenicity of Yersinia *isolates*

Gerbils

Most of the *Yersinia* isolates failed to establish infection in gerbils. Systemic infection, which in many cases was lethal, was produced by nine isolates (Table 2).

Table 2. *The properties of* Y. enterocolitica *and related strains isolated from farm animals*

Source		Reference no.	Species	Biotype	'O' serogroup IP	'O' serogroup W	Phage type	Pathogenicity for gerbils	Nos. of isolates
Bovine	Abortion	47/78	*Y. enterocolitica*	4	NT	5b	NT	+	1
	Faecal/caecal								
		820E	*Y. enterocolitica*	Atypical	10	NT	X_z	+	1
		1167Y	*Y. enterocolitica*	1	$10K_1$	NT	X_o	–	1
		1172Y	*Y. enterocolitica*	Atypical	NT	NT	NT	–	1
	Lymph nodes	2417E	*Y. pseudotuberculosis*	Atypical	NT	NT	NT	–	1
		2543E	*Y. enterocolitica*	1	5	NT	X_o	+	1
		2594E	*Y. enterocolitica*	1	7, 8	7	X_z	–	1
Ovine	Abortion	S.62 (2565)	*Y. enterocolitica*	3	5	5b	X_z	+	1
	Enteritis	474	*Y. enterocolitica*	5	2a, 2b, 3	2, 3	XI	+	1
Porcine	Faecal/caecal	739M		1	6	6(4)	X_z	+	1
		739Y		1	6	6	X_z	+	1
		1317E		1	6	6	X_z	–	
		2066Y		1	6	6	X_z	–	3
		1992Y		1	6	6	X_z	–	
		560SS		1	$10K_1$	NT	X_o	–	
		650M		1	$10K_1$	NT	X_o	–	
		560Y		1	$10K_1$	NT	X_o	–	4
		642M	*Y. enterocolitica*	1	$10K_1$	NT	X_o	–	
		1577Y		1	6, 30	13, 15	X_z	+	1
		2040Y		1	7, 8	7	X_o	–	1
		1454Y		1	27	5a, 13, 19	X_z	+	1
		1510Y		1	27	5a, 13, 19	X_o	–	
		1434Y		1	27	5a, 13, 19	X_o	–	2
		1507Y		1	27	NT	X_o	–	1
		1513Y		1	27	13, 19	X_o	–	1
		1339E		2	NT	10	X_z	–	1
		1506M		Atypical	NT	15, 23, 26	X_o	–	1
		1432Y	*Y. frederiksenii*	.	7, 8	NT	X_z	–	1
		1488Y	*Y. frederiksenii*	.	4, 32, 16	25	X_z	–	1
		715Y	*Y. intermedia*	.	7, 8	5a	X_o	–	1
		562Y	*Y. intermedia*	.	K_1	7, 13	X_z	–	1
		1567Y	*Y. intermedia*	.	17	17	X_o	–	1
		1944Y	*Y. intermedia*	.	NT	NT	X_o	–	1
		564SD	*Y. intermedia*	.	NT	13	X_z	–	1
		562E	*Y. intermedia*	.	AUTO	23	X_o	–	1
		1439Y	*Y. kristensenii*	.	NT	NT	X_o	–	1
		—	*Y. enterocolitica*	NT	ND	ND	ND	ND	12

NT, not typed. ND, not done. AUTO, strains exhibited autoagglutination. IP, Institut Pasteur; W, Winblad; the systems used are not identical and are not intended for comparison.

The course of the infection and the nature and distribution of lesions produced were as described previously for *Y. enterocolitica* 0:9 strain 296/68 (Corbel, 1981).

Mice

None of the *Yersinia* isolates produced enteritis in mice. Furthermore, none of the strains established either local or systemic infection when administered in drinking water. None of the mice yielded yersinias when examined 14 days later.

Serény test

None of the isolates produced any sign of local or general disturbance after instillation onto the conjunctivae of guinea-pigs. The organisms were rapidly cleared from the inoculated eyes without establishing conjunctivitis. All of the animals gave negative results on cultural examination 7 days after inoculation.

Auto-agglutination and growth inhibition on oxalate medium

None of the *Yersinia* isolates auto-agglutinated when grown in tissue culture medium at 37 °C. Only strain 2417E was inhibited when grown on magnesium oxalate medium. Strains 474 and S.62 were also inhibited on oxalate-containing medium when examined soon after isolation but this property was lost on subsequent sub-culture.

DISCUSSION

The much greater frequency of isolation of *Yersinia* strains from pigs compared with cattle was consistent with the results of similar studies conducted in other countries (Ahvonen, Thal & Vasenius, 1973; Alonso *et al.* 1979). These have indicated that *Y. enterocolitica* and related *Yersinia* species are not commonly associated with disease or commensal infection in cattle.

In contrast, these organisms have been found frequently in pigs. In some reports substantially higher rates of infection have been recorded, particularly with human pathogenic serogroups, than were encountered in the present study (Wauters, 1979). This may reflect true differences in the prevalence of *Y. enterocolitica* infection in the porcine populations studied or it may be related to differences in the types of sample and isolation procedures used. Our results are comparable with the findings of a similar study by Hurvell, Glatthard & Thal (1979).

The *Yersinia* strains isolated from pigs in the U.K. did not include the serogroups 0:3 and 0:9 most frequently associated with human yersiniosis in Europe. Most of the isolates belonged to serogroups which are not usually implicated in disease. Nevertheless there were several strains recovered of the 0 serogroups 6, 10 and $10K_1$. These have occasionally been isolated from cases of enteritis in man, although their pathogenicity is uncertain (Alonso *et al.* 1979).

The biotypes and phage types of the porcine isolates were those usually associated with non-pathogenic, commensal or environmental strains in Europe, and this was reflected in the results of the pathogenicity tests.

Because of the small number of *Yersinia* strains isolated from bovine sources it was not possible to draw any definite conclusions about the distribution of particular serogroups or biotypes in cattle in this country. Nevertheless, two of the isolates belonged to the 0:5b (5, 27) serogroup, rare in Europe although quite

commonly associated with human infection in North America (Caprioli, Drapeau & Kasatiya, 1978; Toma, Lafleur & Deidrick, 1979). Interestingly, one of the ovine isolates was also of this serogroup. Two of these three strains were cultured from abortion material, which suggested they were pathogenic. None of the strains showed a high order of pathogenicity according to the criteria used by other authors (Schiemann, Devenish & Toma, 1981). Some loss of virulence may have occurred since isolation, as one of the 0:5b strains was restricted on magnesium oxalate medium soon after isolation, although it became resistant on later sub-culture. This, and other strains, were pathogenic for gerbils although they did not show the other properties which have been correlated with pathogenicity in *Y. enterocolitica*. In particular, none of the strains auto-agglutinated under the conditions specified by Laird & Cavanaugh (1980).

Some of these properties are determined by plasmids (Gemski, Lazére & Casey, 1980) which may be lost or modified on sub-culture, particularly under unfavourable conditions (Portnoy & Falkow, 1981). This may happen frequently to *Y. enterocolitica* isolates *in vitro* and could explain the failure to demonstrate pathogenicity or its correlates in strains obtained from cases of disease in this and other studies.

The failure to find *Y. enterocolitica* strains of serogroup 0:9 which shares antigenic determinants with *Brucella* species (Corbel & Cullen, 1970), is notable. This was not attributable to inadequacy of the methods used, as tests with stock cultures showed that these organisms could be recovered from contaminated material. These results suggest that infection with this serogroup is rare in cattle and pigs in the U.K. and thus an inferquent cause of false-positive cross-reactions to *Brucella*.

The isolation of a strain of *Y. frederiksenii* carrying the 0:16 antigen, strain 1488Y, may be more significant in this respect as this serogroup has also been reported to cross-react serologically with *Brucella* (Nielsen, Ruckerbauer & Samagh, 1981).

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