J. Hyg., Camb. (1984), **92**, 365–375
Printed in Great Britain

Aeromonas hydrophila in livestock: incidence, biochemical characteristics and antibiotic susceptibility

By S. J. GRAY

Public Health Laboratory Service, University Hospital of Wales, Heath Park, Cardiff CF4 4XW

(*Received* 18 *April*; *accepted* 12 *December* 1983)

SUMMARY

Faecal samples from 110 horses, 115 pigs, 111 sheep and 123 cows were examined for the presence of *Aeromonas hydrophila*, which was also sought in the available drinking water.

The overall faecal rate was 11·8%, but significantly more bovine than other samples were found to be positive. There was significant association between the isolation of *A. hydrophila* from all animal faeces and its presence in drinking water, but this was not found when individual animal groups were analysed separately. An enrichment technique increased the total number of isolates by 77·1%. Strains of differing origins could not be differentiated by biotyping, although fermentation of sorbitol was associated with bovine isolates. There was a strong positive correlation between positive reactions for V–P, gluconate oxidase and haemolysis of rabbit erythrocytes, tests which had previously been shown to correlate with production of enterotoxin and cytotoxin. Biotypes giving positive reactions for these tests were most frequently isolated from cows, sheep and untreated water, and less frequently from pigs and horses. Most strains of *A. hydrophila* were resistant to amoxycillin, carbenicillin and cephradine, and sensitive to gentamicin, chloramphenicol and neomycin.

INTRODUCTION

Species of the genus *Aeromonas* have long been recognized as pathogens in amphibians (Emerson & Norris, 1905; Shotts *et al.* 1972), reptiles (Marcus, 1971; Shotts *et al.* 1972), fish (Haley, Davis & Hyde, 1967; Esch & Hazen, 1978) and snails (Mead, 1969).

In humans, it is only in the last decade that *A. hydrophila* has been recognized as an opportunistic pathogen in debilitated or immunologically compromised patients (Ketover, Young & Armstrong, 1973; Davis, Kane & Garagsi, 1978; Cookson, Houang & Lee, 1981). There have been increasing reports of systemic infections in apparently normal individuals (Von Graevenitz and Mensch, 1968; Trust & Chipman, 1979), particularly infections after contact with water (Hanson *et al.* 1977; Davis, Kane & Garagsi, 1978; Fulghum, Linton & Taplin, 1978) or bacteraemia in a burns patient after water contact (Ampel & Peter, 1981). However, the most frequent reports have concerned the involvement of

A. hydrophila in acute diarrhoeal disease (Chatterjee & Neogy, 1972; Bhat, Shanthakumari & Rajan, 1974; Cumberbatch *et al.* 1979; Ljungh, Wretlind & Wadstrom, 1978; Dubey & Sanyal, 1979) in several countries (Trust & Chipman, 1979).

Enterotoxin production by *A. hydrophila* has been demonstrated both in human and animal isolates (Sanyal, Singh & Sen, 1975; Annapurna & Sanyal, 1977). Although Cumberbatch *et al.* (1979) correlated production of a cytotoxin with enteropathogenicity, Ljung, Wretlind & Mollby (1981) showed enterotoxin and cytotoxin to be separate entities.

There appears to be a correlation between enterotoxin and cytotoxin production and certain biochemical tests. Haemolysis of rabbit and human erythrocytes (Ljungh, Popoff & Wadstrom, 1977), production of acetoin (V–P) in non-arabinose fermenters (Burke *et al.* 1982) and gluconate oxidase production J. V. Lee (personal communication) were correlated with production of an enterotoxin. Cytotoxin production was shown to correlate with positive tests for lysine decarboxylase and V–P (Cumberbatch *et al*, 1979) and haemolysis of human and rabbit erythrocytes (Dailey *et al.* 1981).

There is little information on the incidence of *A. hydrophila* in mammals, other than man. Wohlgemuth, Pierce & Kirkbride (1972) isolated it from a case of bovine abortion and Sanyal, Singh & Sen (1975) isolated it from the faeces of a calf. Annapurna & Sanyal (1977) isolated *A. hydrophila* from faeces of domestic animals – cow, buffalo, goat and chickens – although no large-scale study has been reported to date.

Lack of information about the incidence of *A. hydrophila* in domestic animals prompted an investigation of (i) its occurrence in livestock and their drinking water, examined by direct plating and an enrichment technique; (ii) differentiation of isolates of different origins by biotyping, and the relationship of these biotypes to those associated with enteropathogenicity in man; and (iii) the antibiotic susceptibility.

The taxonomy of the genus *Aeromonas* is confused (Kaper, Lockman & Colwell, 1981). In this study isolates which were motile, grew at 37 °C and which did not produce a brown pigment were considered to be *A. hydrophila*.

MATERIALS AND METHODS

Sampling

Seventeen establishments (farms, horse studs, stables and piggeries) were located in south east Wales in the counties of Mid and South Glamorgan. A single farm was located in Carmarthen, West Wales. Samples were collected from 459 animals between May and the end of November as Hazen (1979) had reported optimal densities of *A. hydrophila* in water during this period. This seasonal occurrence was also observed in human faecal isolates by Gracey *et al.* (1982). At each site visited a 300 ml sample of the available drinking water was also collected in a sterile water sampling bottle which contained 0·3 ml of a 1·8 % (w/v) solution of sodium thiosulphate. The pH of the water was determined with a digital pH meter (PT 1–15 model: Data Scientific P.O. Box 19, Princes Risborough, Bucks HP17 9PH) after bacteriological procedures had been completed.

Freshly deposited faecal samples from each animal were collected. Samples collected for horse, sheep and cow were each representative of a single animal, but faeces from pigs were always contaminated with slurry from other pigs sharing the pen.

Isolation media

Xylose deoxycholate citrate agar (XDCA) as selective plating medium, and alkaline peptone water (APW) as enrichment broth were used to isolate *A. hydrophila* (Shread, Donovan & Lee, 1981).

Isolation protocol

Samples were examined as soon after collection as possible, within 2 h at 16 of the establishments visited, and within 24 h of sampling in Carmarthen, where water and faeces were refrigerated overnight.

Faecal samples were inoculated on to XDCA directly, and incubated aerobically at 37 °C for 18 h. Approximately 2 g faeces was inoculated into 25 ml of APW, which was then incubated at 20 °C for 48 h. Subcultures on to XDCA were made from the upper film of growth in APW after 24 and 48 h enrichment. Plate subcultures were incubated as above.

Well-mixed water samples were inoculated directly on to XDCA using one standard loopful which delivered 5 μl. From this the number of aeromonas in the drinking water was estimated. A 50 ml volume of water was then passed through a cellulose acetate membrane filter (Oxoid 'Nuflow': 50 mm diameter, Grade 0·45 μm, code N50/45G). The filter disk was immersed in 25 ml APW and treated as for faecal samples.

Direct and enrichment XDCA were examined for non-xylose fermenting colonies and all morphologically different types were subcultured on horse blood agar.

Presumptive identification

The horse blood agar cultures were tested for oxidase production by the method of Kovacs (1956).

Oxidase-positive organisms were screened with the following tests: motility; Hugh and Leifson (O & F); growth (and fermentation of lactose) on CLED agar; sensitivity to 2,4-diamino-6,7-diisopropyl-pteridine (vibriostatic agent 0/129, 10 μg and 150 μg disks). Motile, fermentative organisms which grew on CLED and were resistant to 10 and 150 μg disks of vibriostatic agent 0/129 were presumptively identified as *A. hydrophila*.

Confirmation of the presumptive identification was made using the API 20E system, incubated at 37 °C for 24 h.

Additional biochemical tests

(i) Gluconate oxidase: 3 ml of gluconate broth (Shaw & Clarke, 1955) was inoculated with the test strain and incubated aerobically at 30 °C for 48 h. A 1·5 ml sample of the broth culture was then transferred to a clean bijou bottle and a clinitest tablet added (Carpenter, 1961). Development of a blue colour with a white precipitate was considered a negative reaction, development of a green or brown colour with a brown, orange or tan precipitate was considered a positive reaction.

Table 1. *Isolation of* A. hydrophila *from animal faeces and available drinking water*

Establishment	Month sampled	*Animals sampled	Water supply †Type	pH	Direct culture ‡cfu/ml	§Enrichment	Faeces positive (%)
1	May	H	U	7·1	600	+	0
2	May	H	U	7·17	< 200	+	0
2	May	H	T	10·17	< 200	−	0
3	June	H, S	T	5·86	< 200	−	0, 0
3	June	C	U	7·16	< 200	−	10·8
			U	7·96	400	+	
4	July	C	U	8·11	400	+	0
			U‖	7.79	600	+	
			T	10·60	< 200	−	
4	July	H	U‖	7·79	600	+	0
¶5	July	S	U	8·10	600	+	66·7
			U	6·97	200	+	
		C	U	7·07	400	+	40·5
6	August	S	U	7·10	< 200	+	16·7
7	September	P	U	6·77	600	+	4·5
		C	U	6·77	200	−	15·2
		H					0
8	October	S, H, C	U	7·35	600	+	0, 0, 0
9	October	P, H	T	7·30	< 200	+	23·1, 75·0
10	October	P	T	7·25	< 200	−	13·0
11	October	P	T	7·30	< 200	−	2·9
12	November	H	T	7·20	< 200	+	7·7
13	November	H	T	7·30	< 200	−	7·7
14	November	H	U	7·27	< 200	+	20·0
15	November	S, H	U	7·19	< 200	+	20·0, 0
16	November	H	T	7·40	< 200	−	0
17	November	H	T	7·27	< 200	−	0

* H, Horse; S, Sheep; C, Cow; P, Pig.
† U, Untreated (stream, river or well water); T, chlorinated.
‡ Colony-forming units (cfu)/ml.
§ +, Positive; −, negative.
‖ These two untreated waters were one and the same.
¶ Establishment 5: water stored for 24 h before examination.

The remaining 1·5 ml of cultures giving a negative reaction was reincubated for a further 48 h before retesting. *Escherichia coli* NCTC 10418 and *Klebsiella aerogenes* were used as negative and positive controls respectively.

(ii) Haemolysis of horse, human and rabbit erythrocytes (7 % v/v erythrocytes in Columbia agar) aerobically at 30 °C for 24 h.

Antibiograms

Sensitivity tests were performed with Oxoid 'Multodisc' rings (Code 6165E) containing the following concentrations of antibiotic (μg) amoxycillin, 10; ceph-

Table 2. *Isolation of* A. hydrophila *from animal faeces*

Animal	Total sampled	Number (%) positive
Horse	110	7 (6·4)
Pig	115	11 (9·6)
Sheep	111	10 (9·0)
Cow	123	26 (21·1)
Totals	459	54 (11·8)

radine, 30; gentamicin, 10; tobramycin, 10; carbenicillin, 100; tetracycline, 10; trimethoprim, 1·25; sulphamethoxazole, 25. Additional neomycin (10) and chloramphenicol (25) disks (Mast Laboratories) were also included with eact test. A control sensitivity test employing *E. coli* NCTC 10418 was set up with each batch of sensitivity tests. Tests were incubated overnight aerobically at 37 °C. Each new batch of medium was controlled with *A. hydrophila* NCTC 8049.

RESULTS

Analysis of the data presented in Table 1 showed that, overall, there was a significant correlation between isolation of *A. hydrophila* from animal faeces and its presence in the available drinking water ($P < 0{\cdot}02$, chi squared with Yates correction). In individual animal groups, however, this correlation disappeared.

A. hydrophila was isolated more frequently from unchlorinated water (14 of 15 positive) than chlorinated supplies (two of 10 positive). Most establishments at which cows and sheep were sampled had untreated water as the available drinking water. Half the establishments at which horses were sampled had chlorinated mains water and half an untreated water supply. The majority of pigs had access to chlorinated supplies only. Numbers of *A. hydrophila* in drinking water did not vary greatly throughout the sampling period.

Of 459 animal faecal samples examined, 54 (11·8%) were positive for *A. hydrophila* (Table 2). Aeromonas was isolated more frequently from cows than from other animal groups sampled ($P < 0{\cdot}0005$), whilst horses had the lowest rate of isolation.

With the exception of two equine faecal samples, all exhibited the normal consistency of stool associated with the healthy animal. Two horses with 'scours' were negative for aeromonas.

Isolation by direct plating alone and an enrichment technique were compared (Table 3). Enrichment greatly increased the number of positive isolates, as 77·1% of total isolates were recovered on enrichment only. Enrichment for 48 h was found to be particularly effective, the extra 24 h yielding a further 58·1% of isolates. Direct plating only proved effective for water samples.

The 54 positive faecal samples yielded 57 strains of *A. hydrophila*, and the 16 positive water samples 28 strains. The 85 strains isolated were uniformly positive for ONPG (β-galactosidase), arginine dihydrolase, gelatin liquefaction, acid production from glucose, and haemolysis of horse and human erythrocytes. Ornithine decarboxylase, H_2S (from thiosulphate), urease, tryptophane deaminase, and fermentation of inositol gave uniformly negative reactions. All isolates were sensitive to gentamicin, tobramycin, neomycin and chloramphenicol.

Table 3. *Method of isolation: comparison in numbers (%) of positive cultures*

Sample	Total positive	Number (%) positive: Direct plating	Direct plating only	Enrichment: 24 h	Enrichment: 48 h	Enrichment only
Horse faeces	7	1 (14·3)	0 (0)	7 (100)	7 (100)	6 (85·7)
Pig faeces	11	1 (9·1)	0 (0)	7 (63·6)	11 (100)	10 (90·9)
Sheep faeces	10	3 (30·0)	0 (0)	4 (40·0)	10 (100)	7 (70·0)
Cow faeces	26	1 (3·9)	0 (0)	11 (42·3)	25 (96·2)*	25 (96·2)
Water	16	10 (62·5)	1 (6·3)	14 (87·5)	15 (93·8)	6 (37·5)
Totals	70	16 (22·9)	1 (1·4)	43 (61·4)	68 (97·1)	54 (77·1)

* One faecal sample positive at 24 h only.

Table 4. *Most common biotypes of* A. hydrophila

Character	Biotypes: 1	2	3	4	5	6	7
Lysine decarboxylase	—	—	—	—	—	—	—
Citrate	0·17	0·08	—	—	0·25	—	—
Indole	+	+	+	+	+	+	0·93
V–P	—	—	—	—	+	0·57	0·93
Gluconate oxidase	—	—	0·2	—	+	+	+
Haemolysis rabbit erythrocytes	—	—	+	+	+	+	+
Acid from mannitol	+	+	+	+	+	+	+
Sorbitol	—	—	—	—	—	—	+
Rhamnose	—	—	—	—	0·25	0·09	—
Sucrose	+	+	+	0·25	+	+	+
Melibiose	—	0·08	—	—	—	—	—
Amygdalin	+	+	0·8	+	—	0·43	0·6
(L+)arabinose	+	0·23	—	+	+	0·04	0·53
Lactose	0·33	0·23	0·4	—	—	—	—
Antibiotic susceptibility							
Amoxycillin	R	R	R	R	R	R	R
Cephradine	R	0·77	R	R	0·5	0·7	0·93
Carbenicillin	R	R	R	R	R	R	R
Tetracycline	S	S	0·2	S	S	S	S
Trimethoprim	R	S	S	0·25	S	S	S
Sulphamethoxazole	R	S	S	0·25	S	S	S
Number of strains	6	13	5	4	4	23	15

Key: +, uniformly positive reactions; —, uniformly negative reactions; R, uniformly resistant; S, uniformly sensitive.

Number < 1, ratio of strains positive or resistant.

Variable characters were used to biotype the 85 isolates of *A. hydrophila* using a simple matching coefficient with UPGMA sorting, at an 88·2 % similarity level. Seventy strains (82·4 %) fell into seven common biotypes (Table 4). Analysis of these data showed that water and pig isolates were not associated with any particular biotype but were dispersed throughout. Of the cow isolates 37·9 % were found in biotype 7 but, with one exception, these cow isolates were from a single

establishment. The remainder could not be assigned to any particular biotype. Biotype 7 contained all those isolates producing acid from sorbitol. Isolations were from three establishments, (*a*) 11 isolates – 10 cow, one sheep; (*b*) three isolates – two untreated waters, one cow and (*c*) one chlorinated water. Six of seven horse isolates were found in biotypes 2 and 6 (three in each), though numbers were small. Of the sheep isolates 70 % were in biotype 6 (three different establishments), although these accounted for only 30·4 % of isolates in this biotype. Isolates which were able to utilize citrate as the sole carbon source were predominantly of water and pig origin, but represented only 21·4 % and 27·3 % of total isolates in the two groups respectively. Also they were not associated with each other or confined to any particular biotype.

The relationship between biochemical tests previously reported to correlate with production of an enterotoxin/cytotoxin was examined. Significant correlation was shown between tests for V–P and gluconate oxidase ($P < 0{\cdot}001$ by chi squared), V–P and haemolysis of rabbit erythrocytes ($P < 0{\cdot}001$), and gluconate oxidase and haemolysis of rabbit erythrocytes ($P < 0{\cdot}001$). On the basis of these correlations, V–P-positive isolates were more likely to give positive reactions for gluconate oxidase and haemolysis of rabbit erythrocytes than V–P-negative isolates. All isolates haemolysed human erythrocytes. V–P-negative isolates were equally as likely to be arabinose negative as V–P-positive isolates. As only three isolates produced lysine decarboxylase there was no association with any of the other biochemical markers of toxigenicity.

Positive reactions for V–P, gluconate oxidase and haemolysis of rabbit erythrocytes were found in 60 % of sheep, 53·6 % of water and 51·7 % of cow isolates, whereas only 36·4 % of pig 14·3 % of horse isolates gave such reactions.

Most isolates were resistant to amoxycillin (97·6 %), carbenicillin (94·1 %) and cephradine (71·8 %). Smaller numbers were resistant to sulphamethoxazole (16·5 %), trimethoprim (10·6 %) and tetracycline (4·7 %).

DISCUSSION

The incidence of *A. hydrophila* in the healthy animal population is higher than the asymptomatic faecal carriage rate in humans (Von Graevenitz & Zinterhofer, 1970; Shread, Donovan & Lee, 1981; Gracey *et al.* 1982). However, Pitarangsi *et al.* (1982), in the indigenous Thai population, found no significant difference between faecal isolation rates of *A. hydrophila* in symptomatic (9–34 %) and asymptomatic (8–27 %) individuals. There is evidence that human infections associated with *A. hydrophila* are a result of exposure to water containing these organisms (Phillips, Bernhardt & Rosenthal, 1974; Fulghum, Linton & Taplin, 1978; Cumberbatch *et al.* 1979; Ampel & Peter 1981; Pitarangsi *et al.* 1982).

The high incidence of *A. hydrophila* in apparently healthy animals may, therefore, only reflect constant exposure to water containing the organism. This would appear to be substantiated by the significant overall correlation found between its presence in animal faeces and the available drinking water. However, this association was not seen in the individual animal groups. Indeed, this apparent overall association was in the main due to the greater number of positive cow faeces found with positive waters, as there was no overall statistical correlation between

faecal isolates and positive water supplies in the other three animal groups ($P < 0.5$).

The cow, sheep and horse groups studied were by nature 'free' roaming animals with ready access to untreated water with a high likelihood of contamination with *A. hydrophila*. However, modern intense methods of pig farming mean that these animals have, in the majority of instances, only access to chlorinated supplies, in which one would not expect to find *A. hydrophila*. Yet the isolation rate in pigs was similar to that seen in sheep and greater than that found in horses. It is possible that even though a water supply contaminated with *A. hydrophila* may contribute to its subsequent faecal isolation, some other vehicle of entry into the intestine may also be important.

The significantly greater isolation rate in cows may, at least in part, be due to an epiphenomenon. Eighteen of 26 (69·2 %) isolates from cow faeces were from a single establishment, although the total number of cows sampled there represented only 34·1 % of total cows sampled. Further, four of 10 (40 %) positive sheep were from the same establishment, even though the numbers sampled represented only 5·4 % of total sheep samples. Thus the significantly higher isolation rate in cows may not be all that it appears. Indeed, isolation rates for cows would equal those of pigs, and the rate of isolation in sheep would closely approximate the rate in horses if the samples from this establishment were ignored (9·9–9·6 % and 5·7–6·4 % respectively).

The advantage of employing an enrichment technique was clearly shown, enrichment for 48 h at 20 °C being particularly effective. Shread, Donovan & Lee (1981) first proposed alkaline peptone water (APW) as an enrichment medium for aeromonas, and Von Graevenitz & Bucher (1983) found APW (and xylose–DCA) to have optimal sensitivity and specificity for *Aeromonas* spp., although Moulsdale (1983) found the evidence for its use to be inconclusive. Only in water samples was direct plating useful in isolating *A. hydrophila*, presumably because there were greater numbers in water.

In general *A. hydrophila* isolated from the various animal groups and their water supply could not be assigned to any particular biotype. No single test or group of tests would differentiate between isolates of different origin. Biotype 7 contained all those *Aeromonas* strains producing acid from sorbitol, and 73·3 % of this biotype were isolated from cow faeces. However, as 10 of 11 bovine isolates in this biotype were confined to a single establishment, the association between the two may be due to a localized phenomenon, particularly as the single sheep isolate which fermented sorbitol was also isolated from this establishment.

Sanyal, Singh & Sen (1975) demonstrated enterotoxin production by *A. hydrophila* which led to its increasing incrimination as an enteropathogen. Cumberbatch *et al.* (1979) correlated possession of a cytotoxin with enteropathogenicity. Techniques enabling detection of either toxin are not readily adaptable to routine laboratory use, so there have been several attempts to find a biochemical test (or tests) which correlate with toxin production. Lysine decarboxylase production, and a positiveV–P test, were shown to correlate well with production of a cytotoxin by Cumberbatch *et al.* (1979), Dailey *et al.* (1981) and Kaper, Lockman & Colwell (1981). Burke *et al.* (1982) found good correlation between lysine decarboxylase production and presence of an enterotoxin, and also between

enterotoxin and V–P-positive, arabinose-negative isolates. Ljungh, Popoff & Wadstrom (1977) found good correlation between haemolysis of rabbit and human erythrocytes and enterotoxin production, whilst Dailey *et al.* (1981) found good correlation between these haemolytic systems and production of an enterotoxin and cytotoxin. Burke *et al.* (1982) found that 97 % of enterotoxin-producing strains could be correctly identified by haemolysis of rabbit erythrocytes, whilst Pitarangsi *et al.* (1982) showed 100 % correlation between haemolysis of rabbit erythrocytes and cytotoxin production. J. V. Lee (personal communication) found an 82 % correlation between enterotoxin production and a positive gluconate oxidase test.

Although this study did not investigate toxin production *per se*, analysis of those tests correlating with toxin production was valuable in assessing the environmental reservoir of *A. hydrophila* potentially enteropathogenic to man. Burke *et al.* (1982) found that no single biochemical test differentiated between enterotoxin-positive and negative strains, and any assumption about potential enteropathogenicity could only be made by considering the results of a number of tests. Most isolates of biotypes 5, 6 and 7 gave positive reactions for V–P, gluconate oxidase and haemolysis of rabbit erythrocytes, whereas the majority of isolates forming biotypes 1–4 were negative for these tests. It was interesting to note that gluconate oxidase was principally responsible for splitting the two groups of biotypes. Sheep, water and cow isolates were much more frequently positive for all three tests than were pig or horse isolates. This would suggest that pig and horse isolates were less toxigenic than either cow, sheep or water isolates, and so less of a potential risk to humans.

The correlation Burke *et al.* (1982) found between V–P-positive, arabinose-negative isolates and enterotoxin production was not seen in animal and water isolates ($P < 0{\cdot}20$), if positive reactions for V–P, gluconate oxidase and haemolysis of rabbit erythrocytes were used as markers of potential toxin production. Neither was there correlation between these three tests and haemolysis of human erythrocytes.

No correlation was found between a positive lysine decarboxylase (L.D.C.) test and production of V–P, gluconate oxidase and haemolysis of rabbit erythrocytes. Cumberbatch *et al.* (1979) commented on the variable results of tests for lysine decarboxylase found by various authors. The reproducibility and credibility of this test appears questionable. Cumberbatch suggested that the discrepancy in this test may be overcome if the length of incubation is increased from 24 to 48, or even 96 h, even in the 'rapid' identification API 20E system. Using Moller's method, L.D.C. reactions are not recorded as negative until 96 h have elapsed, and the reaction may vary with temperature of incubation chosen (J. V. Lee, personal communication). In the experience of API Laboratory Products Ltd (personal communication) approximately 50 % of strains give a positive reaction for this test in API 20E. However, recent experience with *A. hydrophila* has shown that a strain may be positive after overnight incubation in Moller's medium, but negative in API 20E after 96 h incubation at both 37 and 30 °C (unpublished data). Thus, until this test becomes more standardized any correlation found between the result and toxin production may be of a purely arbitrary nature. The taxonomic confusion within the genus *Aeromonas* also probably contributes considerably to the diversity of reactions quoted for *A. hydrophila*.

The reservoir of *A. hydrophila* in the healthy animal population and in untreated water may represent a considerable potential source of human infection. Biochemical tests shown to correlate with the potential enteropathogenic effect of the organism suggest that cow, sheep and water isolates may be more of a potential risk as human enteropathogens than those from either pig or horses. As *A. hydrophila* has now been shown to be present in environments other than water, the potential risk to humans working in close contact with livestock must not be disregarded. Whether their presence in the healthy animal is a reflexion of a contaminated water supply is yet to be elucidated.

I would like to thank Dr J. V. Lee for help and encouragement in the production of this work; Dr R. G. Newcombe and Dr T. Bryant for statistical help, and Miss C. A. James for the typescript.

REFERENCES

AMPEL, N. & PETER, G. (1981). *Aeromonas* bacteraemia in a burn patient. *Lancet* ii, 987.

ANNAPURNA, E. & SANYAL, S. C. (1977). Enterotoxicity of *Aeromonas hydrophila*. *Journal of Medical Microbiology* **10**, 317–323.

BHAT, P., SHANTHAKUMARI, S. & RAJAN, D. (1974). The characterization and significance of *Plesiomonas shigelloides* and *Aeromonas hydrophila* isolated from an epidemic of diarrhoea. *Indian Journal of Medical Research* **62**, 1051–1060.

BURKE, VALERIE, ROBINSON, JENNIFER, ATKINSON, H. M. & GRACEY, M. (1982). Biochemical characteristics of enterotoxigenic *Aeromonas* spp. *Journal of Clinical Microbiology* **15**, 48–52.

CARPENTER, K. P. (1961). The relationship of the enterobacterium A.12 (Sachs) to *Shigella boydii* 14. *Journal of General Microbiology* **26**, 535–542.

CHATTERJEE, B. D. & NEOGY, K. N. (1972). Studies on *Aeromonas* and *Plesiomonas* species isolated from cases of choleric diarrhoea. *Indian Journal of Medical Research*. **60**, 520–524.

COOKSON, B. D., HOUANG, E. C. & LEE, J. V. (1981). Clustering of *Aeromonas hydrophila* septicaemia. *Lancet* ii, 1232.

CUMBERBATCH, N., GURWITH, M. J., LANGSTON, C., SACK, R. B. & BRUNTON, J. L. (1979). Cytotoxic enterotoxin produced by *Aeromonas hydrophila*: relationship of toxigenic isolates to diarrhoeal disease. *Infection and Immunity* **23**, 829–837.

DAILEY, O P., JOSEPH, S. W., COOLBAUGH, J. C., WALKER, R. I., MERRELL, B. R., ROLLINS, D. M., SEIDLER, R. J., COLWELL, R. R. & LISSNER, C. R. (1981). Association of *Aeromonas sobria* with human infection. *Journal of Clinical Microbiology* **13**, 769–777.

DAVIS, W. A., KANE, J. G. & GARAGSI, V. F. (1978). Human *Aeromonas* infections: a review of the literature and a case report of endocarditis. *Medicine* **57**, 267–277.

DUBEY, R. S. & SANYAL, S. C. (1979). Characterization and neutralization of *Aeromonas hydrophila* enterotoxin in the rabbit ileal-loop model. *Journal of Medical Microbiology* **12**, 347–354.

EMERSON, H. & NORRIS, C. (1905). 'Red-Leg' – an infectious disease of frogs. *Journal of Experimental Medicine* **7**, 32–60.

ESCH, G. W. & HAZEN, T. C. (1978). *Energy and Environmental Stress in Aquatic Systems.* Department of Energy Symposium series number CONF 771114. (ed. J. H. Thorpe and J. W. Gibbons, National Technical Information Service, Springfield, Virginia).

FULGHUM, D. D., LINTON, W. R. & TAPLIN, D. (1978). Fatal *Aeromonas hydrophila* infection of the skin. *Southern Medical Journal* **71**, 739–741.

GRACEY, M., BURKE, V., ROCKHILL, R. C., SUHARYONO & SUNOTO (1982). *Aeromonas* species as enteric pathogens. *Lancet*, i, 223–224.

HALEY, R., DAVIS, S. P. & HYDE, J. M. (1967). Environmental stress and *Aeromonas liquefaciens* in American and thread fin shad mortalities. *Progressive Fish Culture* **29**, 193.

HANSON, P. G., STANDRIDGE, J., JARRETT, F. & MAKI, D. G. (1977). Freshwater wound infection due to *Aeromonas hydrophila*. *Journal of the American Medical Association* **238**, 1053–1054.

HAZEN, T. C. (1979). Ecology of *Aeromonas hydrophila* in a South Carolina reservoir. *Microbial Ecology* **5**, 179–195.

KAPER, J. B., LOCKMAN, H. & COLWELL, R. R. (1981). *Aeromonas hydrophila*: ecology and toxigenicity of isolates from an estuary. *Journal of Applied Bacteriology* **50**, 359–377.

KETOVER, B. P., YOUNG, L. S. & ARMSTRONG, D. (1973). Septicaemia due to *Aeromonas hydrophila*: clinical and immunologic aspects. *Journal of Infectious Disease* **127**, 284–290.

KOVACS, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature, London.* **178**, 703.

LJUNGH, A., POPOFF, M. & WADSTROM, T. (1977). *Aeromonas hydrophila* in acute diarrhoeal disease: detection of enterotoxin and biotyping of strains. *Journal of Clinical Microbiology* **6**, 96–100.

LJUNGH, A., WRETLIND, B. & WADSTROM, T. (1978). Evidence for enterotoxin and two cytolytic toxins in human isolates of *Aeromonas hydrophila*. In *Proceedings of the 5th International Symposium on Toxins: Animal, Plant, and Microbial* pp. 947–960. (ed. P. Rosenberg), New York: Pergamon Press.

LJUNGH, A., WRETLIND, B. & MOLLBY, R. (1981). Separation and characterization of enterotoxin and two haemolysins from *Aeromonas hydrophila*. *Acta Pathologica et Microbiologica Scandinavica* (*Section B*). **89**, 387–397.

MARCUS, L. C. (1971). Infectious diseases of reptiles. *Journal of The American Veterinary Medical Association* **159**, 1629–1631.

MEAD, A. R. (1969). *Aeromonas liquefaciens* in the leukodermia syndrome of *Achatina fulica*. *Malacologia* **9**, 43.

MOULSDALE, M. T. (1983). Isolation of *Aeromonas* from faeces. *Lancet* i, 351.

PHILLIPS, J. A., BERNHARDT, H. E. & ROSENTHAL, S. G. (1974). *Aeromonas hydrophila* infections. *Pediatrics* **53**, 110–112.

PITARANGSI, C., ECHEVERRIA, P., WHITMIRE, R., TIRAPAT, C., FORMAL, S., DAMMIN, G. J. & TINGTALAPONG, M. (1982). Enteropathogenicity of *Aeromonas hydrophila* and *Plesiomonas shigelloides*: prevalence among individuals with and without diarrhoea in Thailand. *Infection and Immunity* **35**, 666–673.

SANYAL, S. C., SINGH, S. J., & SEN, P. C. (1975). Enteropathogenicity of *Aeromonas hydrophila* and *Plesiomonas shigelloides*. *Journal of Medical Microbiology* **8**, 195–198.

SHAW, C. & CLARKE, P. H. (1955). Biochemical classification of Proteus and Providence cultures. *Journal of General Microbiology* **13**, 155–161.

SHOTTS, E. B., GAINS, J. L., MARTIN, C. & PRESTWOOD, A. K. (1972). Aeromonas-induced deaths among fish and reptiles in a eutrophic inland lake. *Journal of the American Veterinary Medical Association* **161**, 603–607.

SHREAD, P., DONOVAN, T. J., LEE, J. V. (1981). A survey of the incidence of *Aeromonas* in human faeces. *Society for General Microbiology Quarterly* **8**, 184.

TRUST, T. J. & CHIPMAN, D. C. (1979). Clinical involvement of *Aeromonas hydrophila*. *Canadian Medical Association Journal* **120**, 942–947.

VON GRAEVENITZ, A. & Mensch, A. H. (1968). The genus Aeromonas in human bacteriology. Report of 30 cases and review of the literature. *New England Journal of Medicine* **278**, 245–249.

VON GRAEVENITZ, A. & ZINTERHOFER, L. (1970). The detection of *Aeromonas hydrophila* in stool specimens. *Health Laboratory Science* **7**, 124–127.

VON GRAEVENITZ, A. & BUCHER, C. (1983). Evaluation of differential and selective media for isolation of *Aeromonas* and *Plesiomonas* spp. from human faeces. *Journal of Clinical Microbiology* **17**, 16–21.

WOHLGEMUTH, K., PIERCE, R. L. & KIRKBRIDE, C. A. (1972). Bovine abortion associated with *Aeromonas hydrophila*. *Journal of the American Veterinary Medical Association* **160**, 1001–1002.