Some observations on the faecal carriage of mesophilic Aeromonas species in cows and pigs

S. J. GRAY¹ AND D. J. STICKLER²

¹ Public Health Laboratory Service, University Hospital of Wales, Heath Park, Cardiff CF4 4XW.
² School of Pure and Applied Biology, University of Wales College of Cardiff, P.O. Box 13, Cardiff CF1 3XF

(Accepted 22 June 1989)

SUMMARY

Replicate faecal samples from healthy individual pigs and cows were examined for the presence of Aeromonas sp. over a 12-month period. Aeromonads were found to be minor components of the faecal flora, only 8.8% of 520 samples from pigs and 4.6% of 481 samples from cows proving positive. Isolation rates in both groups of animals were seasonal. A. hydrophila (62% of the isolates) was the predominant species in cows, followed by A. caviae (32%) and A. sobria (15%). This pattern was also recorded in the natural waters that the animals drank from during the period when the faecal carriage rate was at its highest. In pigs, A. caviae (59%) was more common than A. hydrophila (41%). A. sobria was not found in any of the pig-associated samples. It seems that cattle acquire their faecal aeromonads from drinking water. The source of the organisms in pigs is less clear.

INTRODUCTION

The mesophilic species of the genus Aeromonas can be isolated from a wide range of aquatic habitats (1–4). They have been long recognized as primary pathogens of cold-blooded animals such as, amphibians, reptiles and fish (5–7). In humans they can act as opportunistic pathogens in debilitated or immunologically compromised patients (8–11). There have also been many reports of the isolation of Aeromonas spp. from cases of human gastro-enteritis when no other recognized enteric pathogen has been found (12–15). Although an attempt to produce enteric disease by administration of aeromonads to human volunteers failed (16) many strains of Aeromonas spp. have been shown to produce enterotoxins and other factors which could endow these organisms with the capability for enteropathogenicity (17–22).

The lack of information on the incidence of Aeromonas spp. in mammals other than man, prompted Gray (23) to search for them in the intestinal flora of cows, pigs, horses and sheep. Analysis of single samples from 459 animals revealed the presence of aeromonads in 21%, 9.6, 6.4 and 9.4% of the faecal samples respectively. As the reservoir of aeromonads in healthy livestock may represent a potential source of human infection it was decided to carry out a more detailed
study of the ecology of these organisms in the agricultural environment. The specific objectives were (a) to observe the faecal carriage rates in cohorts of cows and pigs over a 12-month period; (b) to establish the incidence of the organisms in the environment and the feed and water supplies of the animals over the same period and (c) to examine the relationship between the faecal and environmental isolates.

MATERIALS AND METHODS

Sampling sites
Two adjacent farms situated in the county of South Glamorgan provided the livestock that were sampled during this study, farm A, the pigs and farm B, the cows.

Pig breeding was the main activity on farm A, up to 2000 pigs and piglets being held at any one time. Sampling was facilitated by the farming practice of keeping gilts and sows (Large White-cross-Landrace) in individual Ministry of Agriculture approved metal cages during gestation and farrowing. The gestation or stall house contained a maximum of 112 sows and was separated from the farrowing houses both of which had a capacity of 36 sows. Once weaned in the farrowing house the piglets were put into rearing pens. The sows were then moved into communal pens in the serving area, containing up to five animals, where they were mated with the resident boars. There was thus a continual three house cycle; serving area – 4 weeks; stall house – 3 months; farrowing house – 4 weeks.

In the farrowing house the only water available to the pigs was a chlorinated supply, operated via snout valves in feeding troughs. The diet consisted of feed pellets. No separate water supply was available in the stall and serving areas, food and liquid being obtained from the piped supply of pig swill, the owner being a Ministry of Agriculture approved producer.

Farm B was a mixed farm which maintained a dairy herd of some 250 Friesian-Holstein cows. From November 1985 to the end of April 1986 the herd was kept indoors and fed on a mixed diet of silage and feed concentrates. The cows drank from galvanized steel water troughs supplied with chlorinated mains water. From 1 May 1986 cows were turned out to graze on pasture by day and housed at night. During the day the animals had access to water in drainage channels or ‘reens’ and by night to the chlorinated supply. From the end of May 1986 to the time when sampling ceased in October 1986 cows were permanently out to pasture and had access only to natural reen waters. Cows were fed concentrated feed nuts during milking throughout the year.

Sampling procedures
Faecal samples from pigs and cows were collected at monthly intervals from November 1985 to October 1986 inclusive, although pigs were sampled twice during November 1985. Forty sows and gilts and 40 cows were selected for sampling, pigs being identified by a numbered ear tag and cows by a number branded on their hind quarters. Whenever possible those animals originally selected were sampled at each visit, although this was not always possible. Faeces were collected from the individual pigs when they were located in the stall and farrowing houses, while rectal swabs were obtained from individuals in the
Aeromonas sp. in pigs and cows

communal pens of the serving area. Rectal swabs were obtained from cows at the
time of milking.

Water, feedstuffs, bedding materials and a variety of other environmental
samples were collected throughout the study or as they became available to the
animals. All samples were collected in appropriate sterile containers and examined
within 6 h of sampling. At the beginning of the study a small number of milk
samples were obtained from cows found excreting aeromonads at the previous
sampling.

Isolation protocol

**Faeces.** Isolation and enrichment techniques were as previously described (23),
using Xylose-Deoxycholate Citrate Agar (XDCA) and alkaline peptone water
(APW).

**Waters: enumeration.** Serial tenfold dilutions of chlorinated and unchlorinated
waters (100 ml) were examined by a membrane filtration technique. After
filtration the membrane being applied to the surface of XDCA and incubated at
37 °C for 18 h. Membranes from undiluted waters were also enriched in APW.

**Straw and pasture washings.** Each sample of material (25 g) was washed in sterile
distilled water (200 ml) by violent agitation. Washings were then treated as for
water samples.

**Other samples.** To each sample (20 g) of concentrated cattle and piglet feed
concentrate, silage, silage-feed concentrate mix, stall bedding, sawdust and soil
was added 100 ml APW. One 20 ml volume of pig swill was also treated in this
way, while a second was pre-treated with Tween 80 (20 ml, 1 % v/v) in an attempt
to disperse the fat in the swill, which could possibly have interfered with
enrichment procedures. Small samples of dust and sludge which built up on
window sills in the pig houses were enriched in 25 ml APW. Trapped flies were
immersed in APW (25 ml) and crushed against the side of the container with a
sterile swab. Milk samples (1 ml) were enriched in APW (25 ml) at 20 °C for 48 h.
Enrichment broths were subcultured after 24 and 48 h onto XDCA and incubated
at 37 °C overnight.

**Identification of isolates**

All morphologically distinct non-xylose fermenting colonies were subcultured
onto horse blood agar for further testing. Presumptive identification of isolates as
Aeromonas sp. was as described previously (23). At least 10 % of the total number
of non-xylose fermenting colonies on the membranes were always examined. APW
enrichments from waters and washings shown to contain aeromonads by
membrane enumeration were not processed further.

Confirmation of presumptive identification was by API 20E (API Laboratory
Products, Basingstoke, Hants), a system capable of identification to the generic
level. The identification strips were incubated at 30 °C for 5 days and examined
daily. Indole and V-P production were tested after 48 h.

Isolates identified as Aeromonas spp. were speciated by the criteria of Popoff &
Véron (24) as modified by Lee & Donovan (25). Additionally three enzymes
assayed by the API ZYM kit (API Laboratory Products, Basingstoke, Hants.),
chymotrypsin, β-glucuronidase and β-glucosidase, were used in speciation as these
Table 1. The isolation of mesophilic Aeromonas sp. from the faeces of pigs and cows

<table>
<thead>
<tr>
<th></th>
<th>Pigs</th>
<th>Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals sampled</td>
<td>71</td>
<td>86</td>
</tr>
<tr>
<td>Total number of samples</td>
<td>520</td>
<td>481</td>
</tr>
<tr>
<td>Number of animals colonized with Aeromonas sp.</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td>Number (%) of samples producing Aeromonas sp.</td>
<td>.46(8.8)*</td>
<td>22(4.6)</td>
</tr>
</tbody>
</table>

* Difference in isolation rates from pigs and cows was significant ($\chi^2 = 7.2$, d.f. = 1, $P < 0.01$).

Table 2. A comparison of direct culture and enrichment for the isolation of Aeromonas sp.

<table>
<thead>
<tr>
<th>Number (%) of samples producing Aeromonas sp.</th>
<th>24 h only</th>
<th>48 h only</th>
<th>24 + 48 h only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number positive faeces</td>
<td>Direct culture</td>
<td>Direct culture</td>
<td>Enrichment</td>
</tr>
<tr>
<td>68</td>
<td>1 (1.5)</td>
<td>0 (0)</td>
<td>11 (16)</td>
</tr>
</tbody>
</table>

had been shown to be useful adjuncts to the more frequently used biochemical tests (26).

RESULTS

Faecal isolation rates

Over the 12 month-period faecal samples from 71 pigs and 86 cows were examined for mesophilic aeromonads. A summary of the isolation results is shown in Table 1. All stools examined exhibited the consistency associated with the healthy non-diarrhoeal animal. Aeromonads were isolated more frequently from pig (8.8%) than cow (4.6%) faeces and the difference in isolation rates was significant ($\chi^2 = 7.2$, d.f. = 1, $P < 0.01$). Some of the 68 positive faecal samples produced more than one biotype of aeromonad and a total of 97 faecal isolates were collected.

The majority (98.5%) of faecal samples produced aeromonads only after enrichment (Table 2). In 46% of the positive samples the organism was only detected after 48 h enrichment. However, in 11 faecal samples which were positive after 24 h enrichment, aeromonads were not isolated on subsequent subculture, confirming the value of examining the enrichment broth both at 24 h and 48 h.

Seasonal variation in faecal isolation rates

The seasonal variation in the isolation rates from faeces is presented in Figure 1. In cows the isolation rate was low throughout the year except for a summer peak in August. Isolation rates from pigs were generally higher than from cows, and reached a peak in December. Of the 35 pigs and 20 cows that were positive at some stage of the investigation, aeromonads were recovered in consecutive monthly samples from only 2 of the pigs and none of the cows.
Aeromonas sp. in pigs and cows

Fig. 1. Seasonal variation in the faecal isolation rate of Aeromonas spp. from pigs and cows.

Distribution of Aeromonas sp. in environmental samples

The feed supplies of the pigs on farm A consistently failed to produce any aeromonads (Table 3). The main sources were the slurry of faeces diluted with urine and water and the chlorinated water supply. The ever present population of flies also harboured the organism. Aeromonads were recovered after enrichment from a single sample taken from the outside of a bale of straw, a further nine samples taken from the middle of bales were all negative. The 14 samples of sawdust used as bedding material in the farrowing houses were all negative, as were the nine dust swabs taken from windowsills and the bars of pens.

The results from farm B (Table 4) indicate that cows were similarly exposed to aeromonads present in the chlorinated water supply. When cows were turned out to graze they had access to natural drainage water held in drainage channels or reens, all samples of which contained Aeromonas spp. The organisms were also isolated from the pasture they ate and the soil beneath the pasture. Aeromonas spp. were present in the slurry found in the milking parlour, overwintering shed and farm yard. Stall bedding for the cows in the overwintering quarters comprised sawdust and straw, and was positive on a single occasion. However, the individual components, sampled before use, were always negative. One sample of freshly cut grass from the silage clamp proved positive.

Many of the 57 positive environmental samples produced more than one biotype of Aeromonas sp., and a total of 113 environmental isolates were collected.

Enumeration of Aeromonas spp. in chlorinated water supplies serving farms A and B

The results of the enumeration of Aeromonas spp. on the chlorinated water supplies to the animals are presented in Table 5. The mains supply to the farrowing houses were negative for aeromonads on 12 out of 15 samples. The three positive samples were all from the same farrowing house tap. Counts were either

https://doi.org/10.1017/S0950268800030922 Published online by Cambridge University Press
Table 3. Incidence of Aeromonas spp. in environmental samples from farm A

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Chlorinated water</th>
<th>Straw</th>
<th>Slurry</th>
<th>Flies</th>
<th>Swill</th>
<th>Concentrated feed</th>
<th>Piglet feed concentrate</th>
<th>Environmental dust swabs</th>
<th>Sawdust</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number sampled</td>
<td>33</td>
<td>9</td>
<td>17</td>
<td>12</td>
<td>15</td>
<td>16</td>
<td>10</td>
<td>9</td>
<td>14</td>
<td>135</td>
</tr>
<tr>
<td>Number positive</td>
<td>6</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Number of isolates</td>
<td>6</td>
<td>3</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Isolation rate (%)</td>
<td>18</td>
<td>11</td>
<td>47</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>
very low or aeromonads were only detected upon enrichment. The supply to the pens was negative on 15 of 18 occasions sampled. In two samples aeromonads were isolated only after enrichment of the membranes. The third positive sample, from which *Aeromonas* sp. was isolated in relatively high numbers, was taken from a supply to a feed trough which contained a broth of water and concentrated feed nuts. There was no indication that *Aeromonas* sp. contamination of the water supply to farm A was seasonal.

Three water samples were collected from farm B at each visit. One sample from each of two troughs in the building where the cattle were overwintered together with a sample direct from the mains supply of trough 1. Aeromonads were recovered by direct isolation from the main supply serving trough 1 during July and August. Enrichment was necessary in order to recover organisms from samples taken in February and April (Table 5). Water from trough 1 contained aeromonads throughout the sampling period, while the organisms were only recovered from 2 of the 10 samples from trough 2.

*Aeromonas* spp. in drainage water on farm B

The enumeration of aeromonads in natural drainage water held in reens (Fig. 2) clearly shows a seasonal relationship in the incidence of these organisms. There was a rise in numbers as the weather became warmer, culminating in the August peak. Thereafter, their numbers declined.

Ecological relationship between faecal and environmental aeromonads

Attempts were made to determine the source of the faecal isolates of aeromonads. The management of the pigs on farm A allowed an examination of the relationship of the faecal carriage at different stages of the 20-week breeding cycle. The distribution of positive faecal samples from sows in each of the three

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Number sampled</th>
<th>Number positive</th>
<th>Number of isolates</th>
<th>Isolation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated water</td>
<td>30</td>
<td>16</td>
<td>31</td>
<td>53</td>
</tr>
<tr>
<td>Reen water</td>
<td>12</td>
<td>12</td>
<td>42</td>
<td>100</td>
</tr>
<tr>
<td>Milk</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>Bedding</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Slurry</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>Silage</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Pasture</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>Soil from pasture</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>Flies</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Straw</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Teat water spray</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Teat antibiotic spray</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Concentrate feed</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feed mix (silage + nuts)</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sawdust</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>108</td>
<td>39</td>
<td>91</td>
<td>36</td>
</tr>
</tbody>
</table>
Table 5. Colony-forming units of aeromonads in 100 ml chlorinated water samples over an 11-month period

<table>
<thead>
<tr>
<th>Month sampled</th>
<th>Farm A (pigs)</th>
<th>Farm B (cows)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mains tap FH1</td>
<td>Pen valve FH1</td>
</tr>
<tr>
<td>December</td>
<td>NS</td>
<td>ND (-)</td>
</tr>
<tr>
<td>January</td>
<td>NS</td>
<td>0 (-)</td>
</tr>
<tr>
<td>February</td>
<td>NS</td>
<td>0 (+)</td>
</tr>
<tr>
<td>March</td>
<td>0 (-)</td>
<td>NS</td>
</tr>
<tr>
<td>April</td>
<td>NS</td>
<td>0 (-)</td>
</tr>
<tr>
<td>May</td>
<td>0 (-)</td>
<td>0 (-)</td>
</tr>
<tr>
<td>June</td>
<td>0 (-)</td>
<td>0 (+)</td>
</tr>
<tr>
<td>July</td>
<td>0 (-)</td>
<td>0 (-)</td>
</tr>
<tr>
<td>August</td>
<td>0 (-)</td>
<td>0 (-)</td>
</tr>
<tr>
<td>September</td>
<td>0 (-)</td>
<td>0 (-)</td>
</tr>
<tr>
<td>October</td>
<td>0 (-)</td>
<td>346 (+)</td>
</tr>
</tbody>
</table>

FH1, farrowing house 1; FH2, farrowing house 2; ND, not done; NS, not sampled; Figures, colony-forming units in 100 ml water; (+), (-), indicates success or failure of isolation by enrichment from material on the filter.

Fig. 2. Enumeration of aeromonads in drainage (reen) waters on farm B.
Table 6. Relationship of 46 positive pig faecal samples to site of sampling

<table>
<thead>
<tr>
<th>Location of animals</th>
<th>Total faeces sampled</th>
<th>Number positive</th>
<th>Percentage positive</th>
<th>Proportion (%) of total positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stall house</td>
<td>278</td>
<td>10</td>
<td>3.6</td>
<td>22</td>
</tr>
<tr>
<td>Serving area</td>
<td>81</td>
<td>1</td>
<td>1.2</td>
<td>2</td>
</tr>
<tr>
<td>Farrowing houses</td>
<td>161</td>
<td>35</td>
<td>22</td>
<td>76</td>
</tr>
</tbody>
</table>

Fig. 3. Monthly isolation rates from pig faeces in relation to the three-house cycle.

sites in which they were housed is presented in Table 6, and the location of the positive pigs over the 12-month sampling period illustrated in Fig. 3. It is clear that pigs were more likely to excrete aeromonads in their faeces whilst in the farrowing houses than when situated in either of the other two locations. It was also noted that when the sows were in the farrowing houses their faeces was much softer in texture than normal.

From November 1985 to April 1986 (period A) cows were kept indoors with access to chlorinated water only. Their feed comprised a mixture of silage and feed concentrates. During May (period B) cows were turned out to graze by day but were housed at night. Water consumption was thus a mixture of chlorinated and natural drainage water and feed a mixture of grass, concentrates and silage. From June to the end of the study in October 1986 (period C) the cows were at pasture with feed nuts being offered during milking. The animals had access to natural drainage water only.

During May till October, aeromonads were enumerated in washings from grass cut from fields where cows were grazing, and results related to the total viable counts obtained on the DCA agar. During the 6 months of sampling aeromonads were only recovered after enrichment, and then only when total colony counts exceeded $1.28 \times 10^6$ per 100 ml of washings. The majority (78%) of organisms

https://doi.org/10.1017/S0950268800030922 Published online by Cambridge University Press
recovered from the pasture washings were oxidative, oxidase-positive organisms, pseudomonad-like in nature.

Figure 4 attempts to relate water sources shown to contain aeromonads to faecal carriage rates in cows in each of the three different periods of farming practice. It can be seen that the numbers of aeromonads in the trough water never exceeded $8 \times 10^2$ c.f.u. 100 ml$^{-1}$ and this degree of contamination did not result in high rates of faecal carriage during period A. In the summer months however (period C) there was a strong association between the increase in numbers of aeromonads in the reen water and the faecal carriage of the organisms.

Relationship of species to the source of isolation

Table 7 shows the relationship of the three species of *Aeromonas* to the source of isolation. Of the 93 pig-associated isolates 41 (44%) proved to be *A. hydrophila* and 52 (56%) *A. caviae*. *A. sobria* was not isolated. Of the 117 cow-associated isolates *A. hydrophila* was more frequently found (74/117, 63%) than *A. caviae* (32/117, 27%) and *A. sobria* (11/117, 9.4%). The predominance of *A. hydrophila*
**Aeromonas sp. in pigs and cows**

Table 7. Relationship of Aeromonas species to the source of isolation

<table>
<thead>
<tr>
<th>Source of isolate</th>
<th>A. hydrophila</th>
<th>A. sobria</th>
<th>A. caviae</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig faeces</td>
<td>29 (41)</td>
<td>0 (0)</td>
<td>42 (59)</td>
<td>71</td>
</tr>
<tr>
<td>Cow faeces</td>
<td>16 (62)</td>
<td>4 (15)</td>
<td>6 (23)</td>
<td>26</td>
</tr>
<tr>
<td>Chlorinated water on farm A</td>
<td>3 (50)</td>
<td>0 (0)</td>
<td>3 (50)</td>
<td>6</td>
</tr>
<tr>
<td>Chlorinated water on farm B</td>
<td>24 (77)</td>
<td>0 (0)</td>
<td>7 (23)</td>
<td>31</td>
</tr>
<tr>
<td>Environmental samples from farm A</td>
<td>9 (56)</td>
<td>0 (0)</td>
<td>7 (44)</td>
<td>16</td>
</tr>
<tr>
<td>Environmental samples from farm B</td>
<td>10 (67)</td>
<td>1 (7)</td>
<td>4 (27)</td>
<td>15</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>2 (67)</td>
<td>0 (0)</td>
<td>1 (33)</td>
<td>3</td>
</tr>
<tr>
<td>Reen water from farm B</td>
<td>22 (52)</td>
<td>6 (14)</td>
<td>14 (33)</td>
<td>42</td>
</tr>
<tr>
<td>Totals</td>
<td>115 (55)</td>
<td>11 (5)</td>
<td>84 (40)</td>
<td>210</td>
</tr>
</tbody>
</table>

(62%) over A. caviae (23%) and A. sobria (15%) in cow faecal isolates and the ratios of the three species seems to be reflected in the species make-up of cow environmental isolates. A. sobria was found only in cow faeces and the reen water they drank. A. hydrophila was the predominant isolate from both chlorinated (73%) and reen water supplies (52%), A. caviae being isolated less frequently (27% and 33% respectively). Overall, A. hydrophila was the most frequent isolate (54%) followed by A. caviae (40%) and A. sobria (5-2%).

**DISCUSSION**

In an earlier investigation of the incidence of the A. hydrophilia group in the faeces of livestock, single samples from 123 cows and 115 pigs produced isolation rates of 21 and 9-6% respectively (23). The results from the present study (Table 1) show that over a 12-month period, aeromonads were isolated more frequently from pig (8-8%) than cow (4.6%) faeces. Invariably the aeromonads were only isolated from the faecal samples after enrichment in APW for 24 or 48 h (Table 2) confirming the value of this procedure (27, 28). Millership & Chattopadhyay (29) have reported that extending the enrichment beyond 18 h reduced the isolation rate of aeromonads from artificially seeded faeces. In the current study while it was observed that enrichment broths found to be positive at 24 h were not always positive at 48 h, overall, enrichment beyond 24 h increased the isolation rate by 82%.

The isolation of aeromonads from both animal groups exhibited a seasonal pattern (Fig. 1). The frequency of aeromonad isolation from cows was greatest in August (28%). Similar seasonal patterns have been noted in human faeces (13, 22, 30, 31). In contrast in pigs the frequency of isolation was greatest in December.

In the present study 35 of the 71 pigs and 20 of the 86 cows provided positive samples at some stage of the 12-month period. Examination of the replicated monthly samples from the same animals revealed that repeated isolation of aeromonads from consecutive samples was rare. In the case of pigs the organism...

---

https://doi.org/10.1017/S0950268800030922 Published online by Cambridge University Press
was isolated from just single samples from 26 of the animals. In the remaining nine animals where the organisms were recovered from more than one sample, in only two cases were isolations in consecutive months. All 20 of the cows produced aeromonads from the faeces in just single monthly samples. These results suggest that *Aeromonas* spp. are minor components of the faecal flora of normal cows and pigs. While faecal carriage of these organisms by individual animals seems to be intermittent, aeromonads could of course be present in such small numbers that they were detected only irregularly by the isolation procedures.

George and colleagues (32) reported that in humans, aeromonads persist in the faeces of individuals during diarrhoea, but disappear soon after its resolution. Some authors have suggested that while aeromonads compete poorly with the normal intestinal flora in a healthy gut, the changes in the bacterial populations in the diarrhoeic state allows them to compete successfully and survive (33–35). In the present study none of the animals seemed to be suffering from gastroenteritis at the time of sampling.

Examination of a variety of environmental samples from the farms revealed that the only identifiable major sources of the aeromonads were the chlorinated water supplies to the animals, the natural waters and materials that had been contaminated with animal faeces. On farm A aeromonads were not isolated from any of the samples of pig-swill or concentrated feed-stuffs (Table 3). The results from farm B (Table 4) again indicate the absence of the organism from the prepared feedstuffs. The single sample of silage that proved to be positive for aeromonads was taken when freshly cut grass had been added to the old silage stack. Aeromonads were not isolated from mature silage presumably because it is acidic. Pasture used for the grazing of cows was positive on 2 of 6 occasions that it was sampled, perhaps as a result of faecal contamination.

The presence of aeromonads in milk (Table 4) has been noted by other workers (33, 36, 37). This might result from faecal contamination of the milk whilst obtaining the sample by hand, particularly as later samples from the same cow and two others, obtained using aseptic precautions, proved to be negative.

The water provided for the pigs, a chlorinated supply to the farrowing houses, was only occasionally a source of aeromonads (Table 5). No seasonal pattern in isolations from the chlorinated supply was noted and numbers did not increase with an increase in ambient temperature. This is in contrast to previous studies which had demonstrated such relationships in the aquatic habitat (38, 39). It is thus possible that the presence of the organism in the water samples on farm A was a direct result of environmental contamination or contamination of the trough outlets by the pigs themselves. In this connection Figura & Marri (40) have shown carriage of aeromonads in buccal cavities of pigs and suggested that these organisms may contaminate food by this route. It is thus possible that water from the snout-operated valves was contaminated by oral secretions from the pigs.

Similarly on farm B, it may be that the cows were a source of the aeromonads found in the drinking water troughs. Trough 1, located close to the animal stalls and the more intensively used was also the more heavily contaminated (Table 5). In addition the numbers of aeromonads recovered from trough 1 were highest during the time the cows had access to this water source and declined sharply when the animals were put out to pasture in the summer.
Aeromonas sp. in pigs and cows

Over the period April to October water samples were examined from the drainage channels (reens) on farm B, which were used by the cows as a source of drinking water when they were turned out into the fields for grazing. The results (Fig. 2) show clearly that the population of aeromonads rises sharply to a maximum in August and confirms the results of other studies on natural waters (2, 41).

Analyses of the faecal carriage rates in the animals were carried out in an attempt to explain them in terms of the local farming practices. On farm A, the pigs were put through a three location cycle of serving area, gestation pens and farrowing houses. Analysis of the data presented in Table 6 and Fig. 3 by $\chi^2$ tests confirms a highly significant association between the frequency of faecal isolations and residence in the farrowing houses which was independent of the time of year ($P < 0.001$). It is interesting to note the increased frequency of aeromonads during the farrowing period, a time of apparent stress for the sow. The necessity of stress related factors for systemic infection with aeromonads has been noted for fish (41, 42), cows (43), and rabbits (44).

In the case of cows, the maximum rate of faecal carriage occurred during the period in which the animals were continuously outdoors grazing on pasture and obtaining their water from the natural sources of the reens. This maximum isolation rate also coincided with highest recovery of aeromonads from the reen water (Fig. 4).

There is little published information regarding the incidence of the various species of aeromonads in animal stools. While Figura & Marri (40) found $A. \text{hydrophila}$ (75%) to predominate over $A. \text{caviae}$ (25%) and failed to isolate $A. \text{sobria}$ from tongue swabs and caecal contents of slaughtered swine, other studies have collectively referred to isolates as $A. \text{hydrophila}$ (23, 45–47).

The results presented in Table 7 show that the distribution of the three aeromonad species was clearly different in the two groups of animals. In the case of the pigs $A. \text{caviae}$ was more frequently isolated from the faeces than $A. \text{hydrophila}$ and $A. \text{sobria}$ could not be found. In cow faeces $A. \text{hydrophila}$ was the predominant species followed by $A. \text{caviae}$ and $A. \text{sobria}$. This pattern of species distribution was also observed in the water from the land drainage channels on farm B, the source of drinking water for the animals when the faecal carriage rate was at its highest.

In general, the results of this study are consistent with the hypothesis that cattle acquire their faecal aeromonads from their drinking water. The source of the faecal aeromonads in pigs however was less obvious. The highest rate of aeromonad isolation from faeces occurred when the pigs were in the farrowing houses being fed on concentrates rather than swill and when their only source of water was a chlorinated supply which was rarely contaminated with aeromonads.

ACKNOWLEDGEMENTS

We would like to thank Dr C. D. Ribeiro for his constructive criticism in the preparation of this manuscript and Dr R. Newcombe for statistical analysis.
REFERENCES


Aeromonas sp. in pigs and cows