Review

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Plesiomonas shigelloides - a veterinary perspective

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Abstract
Plesiomonas shigelloides is a Gram-negative, oxidase-positive bacillus and is widely distributed in fresh water. It is part of the normal bacterial flora of fish and some water dwelling reptiles and amphibians. Its role as an enteric commensal in man and other mammals is unclear but it has been isolated from asymptomatic individuals. Its role as an enteric pathogen in man has been established but it is of low pathogenicity and the mechanisms of pathogenesis have not been adequately described. Water, shellfish and fish are the commonest sources of infection to man. In contrast, its role as an enteric pathogen in animals is unknown and the epidemiology of such infections has not been determined. Strains of *P. shigelloides* exhibit antibiotic resistance to penicillins due to β–lactamase production. The bacterium is not fastidious in its culture requirements but may be under reported by veterinary, medical and food laboratories.

Plesiomonas shigelloides is a facultatively anaerobic, Gram-negative, oxidase-positive, non-spore forming bacillus. Most strains are motile, possessing both lophotrichous and peritrichous flagella (1). The organism was first described in 1947 (2) and received its approved naming in 1962 (3). It is the type species, and only species, of the genus *Plesiomonas*. It is closely related phenotypically to the genus *Aeromonas* (plesio - neighbour; monas - to *Aeromonas*), both are members of the family *Vibrionaceae*.

Recent genetic studies have indicated that *P. shigelloides* is closely related to the family *Enterobacteriaceae* (4) and shares a common ancestry with the genus *Proteus* (5). As including *Plesiomonas* in the *Enterobacteriaceae* would require a major revision of the characteristics of the family, its taxonomic classification seems secure for the near future.

NATURAL HABITAT

The primary natural habitat of *P. shigelloides* is fresh water and there are reports of its recovery from rivers, lakes, ponds and sediment in a number of countries (6-17). Although most isolations are in tropical or temperate climates, there is a report of its isolation in a cold climate in Sweden (18).

The recovery of the bacterium and/or the density of its growth in freshwater is dependant upon temperature (7, 10-12), the availability of nutrients and the level of sewage pollution (10). There are a number of reports of the seasonal incidence of *P. shigelloides* where growth is enhanced during the warm season and may be absent during the cold season (7,9,17,19). This was not observed in Zaire (20) which has a tropical climate. Growth temperature studies indicate that most strains of the organism cannot grow below 8 to 10°C (12,21,22).

Within the stable ecology of ponds, the growth of *P. shigelloides* is greatest in the mud at the base of the pond (13,17), is dependant on adequate water oxygenation and is tolerant of high pH (13). This tolerance of high pH reflects an important ecological property.

The bacterium has also been isolated from seawater (23,24) but not by all workers (10). In one study, the survival of *P. shigelloides* in sea water was limited to 22 to 25 hours and 100m from the point of discharge of sewage (24). These findings are supported by an in vitro study, which found that the bacterium grew in broth cultures containing 3 to 5% NaCl. Tolerance of salinity increased with the nutrient status of the growth medium (22).

HOST RANGE

The frequency of isolation of *P. shigelloides* in different species mirrors the probability of exposure to the bacterium in its primary habitat, water, and is greatest in fish then reptiles then mammals then birds (25). It is probable that *P. shigelloides* is part of the normal intestinal flora of freshwater fish and some other water dwelling reptiles and amphibians. Their sporadic presence in animal faeces may be due to transient colonisation following ingestion of fish, water or water-contaminated food (26).

P. shigelloides has been isolated from a wide range of warm and cold-blooded species, other than man (Table 1). Zoological collections are a further source of sporadic isolates including African hunting dogs and wolves in the UK, and cheetah, chimpanzee, Kodiak bear, Black lemur, Black and White Ruffed lemur and Jackass penguins in the USA (C. Furley; personal communication).

P. shigelloides is generally not considered to be a part of the normal faecal flora of man although the bacterium has been isolated from asymptomatic individuals at prevalence rates ranging from 0.01% to 5.5 % (7,27,28). The status of the bacterium as an enteric commensal in other mammalian species is uncertain but in one survey of stray cats and dogs euthanased in Tokyo between 1974 and 1976 the bacterium was isolated from 10.3% of 389 cats and 3.8% of 967 dogs, most of which were asymptomatic (7). In a feeding trial in the UK, the bacterium was isolated from 7 of 12 healthy cats (29).

Fish and shellfish in the natural habitat of *P. shigelloides* act as secondary reservoirs for the bacterium (18). Reported isolation rates from fish are high compared to mammals. *P. shigelloides* was isolated from the intestines of 59% (n=59) of freshwater fish in Zaire (30) and 10.2% (n=246) of freshwater fish in Japan (7). In Japan the isolation of the bacterium from fish mirrored the seasonality of its isolation from water.

Perhaps not surprisingly, most species of birds from which *P. shigelloides* has been isolated either live on fresh water or feed on lake and river fish.

DISEASE IN MAN

Evidence implicating *P. shigelloides* as a human pathogen has been slow to accumulate since its discovery in 1947. This reflects the low incidence of disease in comparison to other enteric pathogens and the low pathogenicity of the bacterium. Its role as an enteric pathogen has been investigated through case reports, comparison of isolation rates from diarrhoeic and healthy individuals, occasional case control studies and occasional volunteer studies. Further evidence has been provided through the response of infected individuals to antibiotic treatment and the recent demonstration of enterotoxins and cytolysins with a possible role in pathogenesis.

There are a number of case reports in the literature of the isolation of *P. shigelloides* from diarrhoeic stools. These are predominantly from tropical and subtropical countries including Bangladesh (52), India (53), Malaysia (54), Taiwan (55) and Thailand (56,57). There are fewer reports from temperate or cold countries but these include the USA (58), Cuba (59), Canada (60) and Finland (61). In these countries, as might be expected, infection is often associated with travel to tropical countries (57,60,62,63). Such “traveller’s diarrhoea” is seen all year round whereas locally acquired infections tend to mirror the seasonality of *P. shigelloides* in the environment (58,60,64). Co-infection with other enteric pathogens is common (60).

Symptoms associated with gastrointestinal infection commonly include diarrhoea, abdominal pain, tenesmus, nausea, lassitude, chills, fever, headache and vomiting (17,49,60,63,65,66). The large bowel is predominantly involved (60). The incubation period ranges from 1 to 9 days (67) and diarrhoeal stools can vary from mucoid through bloody to large volume and watery (68). The average duration of symptoms in untreated cases is 11 days (63). In a significant proportion of cases, infection becomes chronic (60) and the bacterium may be isolated from faeces for more than two months after infection (61). Gastroenteric infection does not require that the host be immunocompromised (63) although a case was recently recorded in an HIV infected patient in the USA (69).

Plesiomonas septicaemia in man is much less common than gastroenteritis with only 21 cases reported in the literature prior to 1996. 81% of the patients were immunocompromised and there was a case mortality of 62%. In most cases the cause of infection was not identified. (70). 12 of the 21 cases were seen in the paediatric age group, predominantly neonates who were believed to acquire the infection perinatally rather than transplacentally. Septicaemia and meningitis commonly occur together in neonates (71). Even less frequent extra intestinal infections include cholecystitis (72), osteomyelitis (73), pancreatic abscess (74) and polyarthritis (75).

There is conflicting evidence regarding isolation rates from diarrhoeic and non-diarrhoeic people. In one case control study in Bangladesh, more isolates were obtained from non-diarrhoeic controls than from diarrhoeic patients (52). In Thailand, *P. shigelloides* was isolated with similar frequencies from people with and without diarrhoea (28). In Zaire, *P. shigelloides* was isolated from the stools of 41 diarrhoeic patients and not from the stools of 841 non-diarrhoeic individuals (76).

There have been a limited number of case control studies that have demonstrated an association between infection with *P. shigelloides* and gastrointestinal disease (60, 63). However, other case control studies have failed to demonstrate an association (52,28).

One early volunteer study failed to induce human disease by either oral or rectal administration of live *Plesiomonas* cultures (38). 36% of 33 volunteers fed a *P. shigelloides* strain that had produced disease in gnotobiotic piglets shed the organism but none became ill (77).

DISEASE IN OTHER SPECIES

The association between infection and disease is less well documented in animals than in humans. There are occasional single case reports in the literature, no reports comparing isolation rates from faeces of animals with diarrhoea and from animals without diarrhoea, and no case control studies.

Isolation of the bacterium has been reported most frequently in association with clinical disease in individual cats (33, 34, 44). This mirrors the higher isolation rate in cats than in other mammalian species (32). An ongoing survey of faecal samples from UK cats has isolated the bacterium from 6.4% of 94 cats with a history of enteric disease and from 3.8% of 52 healthy cats (Jagger;

unpublished data). A larger survey is planned to investigate whether the observed difference in isolation rates is significant.

Isolation from sick animals of other species appears to be less common. The organism has been isolated from the pneumonic lungs of a common otter (40), from dogs with enteritis (7,39), from the livers of ducks and cattle in Zaire (26), from Rock hopper penguins dying around hatching and from a Kodiak bear with violent bloody diarrhoea (C. Furley: personal communication). The bacterium has also been associated with mortality in freshwater fish and turtles in the USA (15) and with the death of a red-throated diver in the UK (44).

Observations on antemortem and post mortem findings in infected animals are given in Table 2.

EPIDEMIOLOGY OF INFECTION

Most human infections with *P. shigelloides* are thought to be waterborne. Such infections tend to show the same seasonality as recovery of the organism from fresh water sources and are often associated with contamination by sewage wastes. Infection may be acquired through drinking water (16,17,78), contact with recreational water, or by consuming food which has been rinsed with contaminated water (79). Inadequately treated drinking water was the suspected source of *P. shigelloides* infection affecting 978 people in Japan (17). One case was recorded in a man swimming in the Mississippi river in the USA (80). *P. shigelloides* has also been implicated as a cause of diarrhoea in people engaging in recreational activities in seawater (23). Poorly chlorinated well water contaminated food with *P. shigelloides* and *Salmonella hartford*, affecting at least 56 people in Livingston County, USA (81). One of the more unusual case reports of Plesiomonas gastroenteritis was in an infant in Missouri, USA, who was bathed in a bathtub into which aquarium water had previously been emptied (82).

Fish, shellfish and crustaceans are further important sources of infection. Cuttlefish (65), salt mackerel (66), oysters (48,83,84) and crab (72) have all been described as causes of food-borne *P. shigelloides* infection. Case control studies have supported associations between infection and the consumption of seafood and untreated water (60), and the consumption of raw shellfish, especially oysters (63). No *P. shigelloides* isolates grow at 5°C and all are destroyed by pasteurization (60°C for 30 minutes) (22), suggesting that inadequate cooking and refrigeration may contribute to food-borne *P. shigelloides* infection.

There is little or no epidemiological information regarding infections in animals. The frequency of isolation of the bacterium from cats in the Czech Republic was only exceeded by the recovery from trout and aquarium fish (32). The source of the bacterium for the Czech cats was not clear; many of the cats from which the bacterium was isolated ate commercial diets and did not have contact with unprocessed fish.

PATHOGENICITY

Gastroenteritis associated with *P. shigelloides* infection in man may be secretory (watery), more invasive and dysenteric resembling colitis, or a subacute/chronic disease lasting from 2 weeks to 3 months (85). Studies have often focused on enterotoxin production (secretory gastroenteritis) but clinical findings in man suggest that enteroinvasion may be more significant than enterotoxin production (60,63). The overall pathogenic potential of *P. shigelloides* is low (86), consistent with the low incidence of disease in man and animals.

Tests for enteropathogenicity have produced variable, inconsistent and often negative results. For example, the Sereny test for invasiveness (keratoconjunctivitis in guinea-pig or rabbit eyes) has consistently produced negative results with strains of *P. shigelloides* (28,63,87-91). One study reported invasion of HeLa cells (87) whilst further studies failed to reproduce this effect (77,92). The exact enteropathogenic mechanisms of this bacterium are still to be determined but some advances have been made:

Enterotoxin

Variable results have been obtained when testing strains of *P. shigelloides* for the production of enterotoxin. Workers have however demonstrated the production of enterotoxin in the rat ileal loop model (93), the rabbit ileal loop model (64,90,91) and in the suckling mouse assay model (64,90,91). Multiple in vivo passages of the bacterium may be required before a positive rabbit ileal loop response is obtained (90,94). Both heat labile (LT) and heat stable (ST) enterotoxins have been partially purified and characterised (95). The LT was biologically active in the rabbit ileal loop model and the ST in both the rabbit ileal loop model and in suckling mice.

The ST enterotoxin demonstrated in the rabbit ileal loop model appears to be a novel enterotoxin which shares no DNA homology with cloned enterotoxin genes of *Escherichia coli* or *Vibrio cholerae* and is activated by heat treatment of the cell filtrate. This activity of the toxin was increased by in vivo passage and rapidly lost when subcultured in vitro suggesting that in vivo induction may contribute to expression of the toxin (94)

Cytolysin

Studies have demonstrated the production of cytolysins by *P. shigelloides* that are cytotoxic to Vero cells (86,89,96), Y1 cells (86,90,97,98), Hep-2 cell lines (86) and CHO cells (77,90,99). Activity against CHO cells was prevented by pre-incubation with cholera antitoxin in one study suggesting that *P. shigelloides* expresses a cholera-like toxin (77). Cytolysins may have a role promoting colonization or invasion of epithelial cells via tissue destruction or inhibition of resident microflora (86).

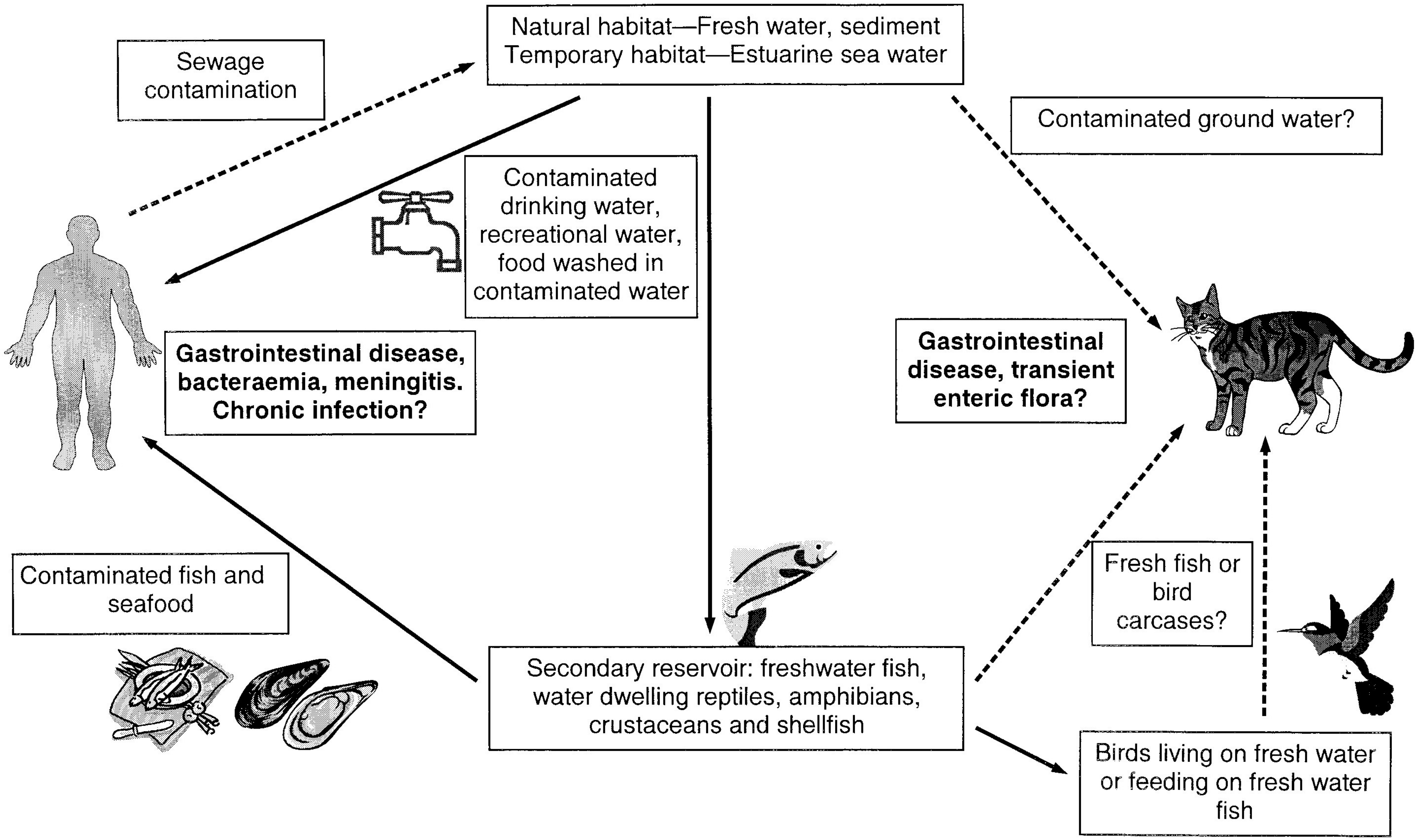

Figure 1. Epidemiology of *Plesiomonas shigelloides* in humans and cats

Haemolysin

Most workers agree that haemolytic activity cannot be detected on rabbit, sheep and bovine blood agars (28, 88). The exceptions are reports of β-haemolysis detected on bovine blood agar (18) and sheep blood agar (59), and reports of α-haemolysis on horse blood agar (100) and unspecified blood agar (55). Haemolytic activity can be detected in some animal strains using a modified CAMP test in which visible lightening is seen of a partial zone of haemolysis associated with *Staphylococcus aureus* growth on blood agar (32). Other workers have demonstrated the production of a cell associated β-haemolysin using gel overlay (101,102) and extracellular β-haemolysin by quantitative assay of a culture supernatant (102,103).

The possible significance of haemolysin production lies in the fact that *P. shigelloides* is able to utilise haemoglobin, hemin and haematin as sources of iron (101,102). Haemolysin activity may release these compounds and this may represent an important means of acquiring iron within a mammalian host. This method of acquisition of iron has also been demonstrated in *Escherichia coli* isolates (104,105) in which it contributes to the pathogenicity of infection. The haemolysin could also help release replicated progeny from invaded cells or act as an enterotoxin in the gut. The activity of the haemolysin does not however correlate with pathogenicity in mice and it is questionable therefore whether it plays a role in systemic infections (101).

Plasmids

Many *Plesiomonas* strains contain a plasmid >120 MDa (63,77,86,89). A large molecular weight plasmid (140 MDa) has been associated with invasiveness of both *Shigella* species and *E.coli* (106). Possession of such a plasmid by *P. shigelloides* did not however correlate with pathogenicity in mice (86) suggesting that it does not facilitate the uptake or invasion of the bacterium in the intestinal tract. However, in a gnotobiotic piglet model, a large molecular weight plasmid appeared to facilitate the uptake of *Plesiomonas* into the mucosa of the distal ileum (92). Other workers have failed to demonstrate any association between specific plasmids and resistance to individual antibiotics or groups of antibiotics (9).

Elastin

Elastin activity was detected in *Plesiomonas* cell suspensions but not in cell culture supernatants using a quantitative elastin-Congo red test system. Elasteolytic enzymes act to degrade connective tissue and can cause extensive tissue damage as well as facilitating the entry of other virulent molecules in vivo (103).

ANTIMICROBIAL RESISTANCE AND THERAPY

Most *P. shigelloides* isolates are resistant to penicillins including ampicillin, piperacillin, ticarcillin, meziocillin and carbenicillin. This seems likely to be due to β-lactamase production by the bacterium (107,108,109), as penicillins combined with a β-lactamase inhibitor are active against isolates in vitro (108). Also, one non b lactamase producing strain of the bacterium was susceptible to all penicillins in vitro (108). A recent study further characterised the β-lactamases produced by some strains of *P. shigelloides* and concluded that β-lactamase was expressed in 50% of the 10 strains tested, that isolates which did not express b lactamase probably had some other mechanism of resistance to penicillins, that environmental isolates expressed a wide range of β-lactamases and that the genes for b lactamase expression in environmental samples existed before β-lactams were developed for therapeutic use (110).

Resistance to aminoglycosides is variable. One study demonstrated that resistance to gentamicin, tobramycin and amikacin was common (111) whereas a second study identified only a few strains with resistance to tobramycin and gentamicin (108). The mechanism for aminoglycoside resistance is not known (85). Plesiomonads are usually sensitive to second and third generation cephalosporins, nitrofurantoin, nalidixic acid, co-trimoxazole, chloramphenicol and quinolones (63, 108, 111).

Based on these findings, suitable agents for treating *Plesiomonas* gastroenteritis include the quinolones and potentiated sulphonamides. The use of aminoglycosides should be avoided. Broad-spectrum penicillins should not be used in extra intestinal infection without antibiotic sensitivity tests on the specific isolate (85). These recommendations can be carried over into veterinary medicine although the sporadic nature of infections in animal species means that in practice, antibiotic sensitivity tests will be performed routinely on isolates.

Antibiotic therapy has been shown to reduce the period to resolution of symptoms in patients with gastroenteritis suggesting that there is a role for antibiotics in intestinal as well as extra intestinal infections (60).

TYPING

Bio typing of this organism is of little value due to the phenotypic homogeneity of the species (112). Minor differences only are observed in e.g. carbohydrate fermentation (59).

Serotyping has been more successful and two major serotyping schemes based on somatic and flagellar antigens were developed to type isolates of *P. shigelloides* (113,114). These schemes dealt predominantly with isolates from man and warm-blooded animals and were recently unified. The unified scheme incorporates 101 somatic (O) antigens and 51 flagellar (H) antigens (115).

A third scheme based on 23 O and 5 H antigens was created with *P. shigelloides* strains originating exclusively from surface water and water insects (14). These antigens have yet to be incorporated into the unified serotyping scheme (115).

Table 1. Isolations of *Plesiomonas shigelloides* by species and country.

Family or species	Country (Reference)
Mammals	
Apes	Other (20, 31)
Binturong	Czech Republic (32)
Cat	UK (29, 31, 33), Norway (34), Czech Republic (32, 35), Japan (7), Other (36)
Chimpanzee	Zaire (20)
Cattle	Zaire (26) Sri Lanka (37)
Dog	Japan (7, 38), UK (31), Czech Republic (32) Germany (39), Zaire (26, 30), Other (36)
Goat	Sri Lanka (37)
Kamtchatka Bear	Belgium (40)
Lemur	Other (41)
Monkey	UK (31) Zaire (20)
Mouse	Czech Republic (32)
Pig	Czech Republic (32), Zaire (26) Japan (43) Other (42)
Polar bear	Belgium (40)
Polecat	Sri Lanka (37)
Raccoon	Czech Republic (32)
Sheep	Sri Lanka (37)
Wolf	Czech Republic (32)
Marine mammals	
Baikal Seal	Belgium (40)
Californian Sea Lion	Belgium (40)
Common Seal	Belgium (40)
Harbour porpoise	UK (44)
Fish	
Aquarium fishes	Czech Republic (32, 45)
Bream	USA (11)
Carp	Czech Republic (32) Japan (7)
Catfish	Czech Republic (32) USA (11)
Crappie	USA (11)
Eel	USA (11)
Grunt	USA (11)
Mullet	USA (45)
Pale Chub	Japan (7)
Pinfish	USA (11)
Pike	Spain (46)
Field Gudgeon	Japan (7)
Ophiocephalus sp.	Zaire (30)
Red tail	Zaire (30)
Sardine	Zaire (30)
Shrimp	USA (11)
Tilapia nilotica	Zaire (30)
Trout	Czech Republic (32) Other (47)
Fresh water fish	Czech Republic (6) Zaire (30), Germany (12) Japan (17) USA (15)
Shellfish and crustaceans	
Clam	USA (11, 45)
Crab	USA (11, 45), Other (48)
Oyster	USA (11, 45), Canada (9)
Shellfish	Japan (17) Other (49)

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Table 1 – continued -

Family or species	Country (Reference)
Reptiles and amphibians	
Crocodile	Czech Republic (32)
Newt	Japan (17)
Snail	Czech Republic (6)
Snake	Other (50)
Tortoise	Czech Republic (32)
Turtle	Czech Republic (6), USA (15)
Xenopus toad	Other (31)
Birds	
Abyssinian Blue-winged Goose	Belgium (40)
Andean Condor	Belgium (40)
Andean Flamingo	Belgium (40)
Black Cormorant	Belgium (40)
Black-faced Ibis	Belgium (40)
Black-footed Penguin	Belgium (40)
Black-headed Gull	Belgium (40)
Brown Pelican	Belgium (40)
Crane	Czech Republic (32)
Domestic fowl	Czech Republic (32)
Duck	Czech Republic (32), Zaire (26)
Egret	Czech Republic (32)
Grey Heron	Belgium (40)
Jabiru Stork	Belgium (40)
James Flamingo	Belgium (40)
Lappet-faced Vulture	Belgium (40)
Lesser Flamingo	Belgium (40)
King Ibis	Belgium (40)
King Penguin	Belgium (40)
Kite	Zaire (26)
Little Penguin	Belgium (40)
Marabou Stork	Belgium (40), Zaire (26)
Ostrich	Czech Republic (32)
Parrot	Czech Republic (32)
Peruvian Penguin	Belgium (40)
Red-throated diver	UK (44)
Turkey vulture	USA (51)

Other, Country of origin not determined.

Table 2. Ante- and postmortem findings associated with *Plesiomonas shigelloides* infection

Family	Ante- and postmortem findings
Mammals (excluding man)	Catarrhal enteritis, splenic tumour, sepsis (32) haemorrhagic diarrhoea (7), pneumonia (40), colitis (33), catarrhal enteritis (34)
Fish	Catarrhal and haemorrhagic enteritis, hepatopancreatic degeneration, ventricular haemorrhage, renal oedema, gall bladder dilation, skin pathology (32), reddened abdomens and injected fins (15)
Reptiles	Liver dystrophy, splenic tumour (32) purulent material in trachea and bronchi (15) ulcerative stomatitis (50)
Birds	Hyperaemia of parenchymatous organs, hepatic degeneration, splenic tumour (32)

It has been recognised for some time that some serotypes of *P. shigelloides* share a somatic antigen with *Shigella sonnei* (phase I O-antigen) (37, 116). This has been of largely academic interest until recently when a *P. shigelloides* strain of serotype O:17 was used to successfully protect rabbits against oral challenge by *Shigella sonnei*, suggesting potential as a vaccine strain for shigellosis in humans (117). A conjugate vaccine of the O-specific polysaccharide from *P. shigelloides* serotype O:17 bound to a bacterial toxoid has been shown to elicit IgG, IgM and IgA antibody responses in adult volunteers (118). A DNA region containing the cluster of genes required for O17 expression has now been successfully cloned in *E. coli* with possible implications for future vaccine development (119).

Plasmid profiles obtained from *P. shigelloides* isolates are heterogenous (89). Potentially this feature could be utilised in epidemiological surveys although there are no reports of such in the literature.

There are as yet no reports of molecular typing studies applied to *P. shigelloides* although it seems likely that PFGE should work (112).

BACTERIAL RECOVERY FROM ENVIRONMENTAL AND CLINICAL SAMPLES

Detection of the organism depends upon tried and tested bacterial isolation techniques. Both enrichment broths and direct plating onto selective media are used. The bacterium has been shown to grow on a wide range of media and at a wide range of temperatures (21, 67). Optimal growth temperatures for most strains occur between 35°C and 38°C (67).

Enrichment broths which are commonly used include alkaline peptone water (120) and tetrathionate broth without iodine. The latter gave consistently greater recovery of *P. shigelloides* from environmental water samples when compared to alkaline peptone water and three other enrichment broths (121). There are however conflicting reports of the usefulness of these two enrichment broths and the choice may depend on the composition of competing flora and the choice of solid plating media (122). Also, incubation at 40°C has been recommended to increase selection for and recovery of *P. shigelloides* (11,121).

Other enrichment broths that have been recommended include nutrient broth with bile salts and brilliant green (123), gram-negative broth (19) and bile peptone broth (124).

One study recovering *P. shigelloides* from aquatic samples by direct plating indicated that both inositol brilliant green bile salts agar and plesiomonas agar should be used in tandem at 35°C, the former because of it's greater recovery rate and the latter because it was less inhibitory for injured bacteria (11). The specificity of the two agars for direct plating of water samples has been shown to be poor but can be improved by applying the oxidase test and 0/129 sensitivity (10). Other workers have recommended the use of *Plesiomonas* differential agar at 44°C to inhibit the growth of *Aeromonas* in environmental samples (125).

Many different selective plating media have been used to isolate *P. shigelloides* from clinical samples including salmonella-shigella agar, modified *Salmonella-Shigella* agar, deoxycholate citrate agar, xylose-lysine-deoxycholate agar, Hektoen enteric agar, deoxycholate lactose agar, MacConkey agar, inositol brilliant green bile salt agar and Endo agar (18,32,125). Inositol brilliant green bile salt agar and *Plesiomonas* differential agar have been recommended for the isolation of both *P. shigelloides* and *Aeromonas* sp. (120).

Opinions differ as to whether the techniques routinely employed in veterinary and medical microbiology laboratories may lead to under-reporting of infection by the bacterium. However, researchers believe that the bacterium can readily be mistaken for an *Enterobacteriaceae* when isolated on non-selective enteric isolation media, unless an oxidase test is performed (63,67). Approximately 30% of strains are reported to be lactose fermenting on enteric agars (107), although this is a variable finding, and these strains may therefore again be mistaken for *Enterobacteriaceae*. Certain enteric media in use in medical laboratories including eosin-methylene blue, brilliant green and *Salmonella-Shigella* agars inhibit the growth of some strains of *P. shigelloides* (107).

SUMMARY

P. shigelloides occupies its own genus in the family *Vibrionaceae* despite being closely related to the *Enterobacteriaceae* (4). It also occupies an environmental niche in fresh water where its growth is dependant upon temperature and the availability of nutrients (7,10-12). Sewage contamination of seawater allows it temporarily occupy estuarine waters (24). Tolerance of high pH probably aids its survival in the environment (13).

The bacterium has been isolated from a wide range of cold and warm blooded species including man. It is not considered a part of the normal enteric flora of man and its status as enteric flora in other mammals is uncertain. It has however been isolated from the stools of asymptomatic people (7,27,28) and from the stools and tissues of asymptomatic cats and dogs (7,29). It probably forms part of the normal bacterial flora of shellfish, fish and some water dwelling reptiles and amphibians.

Evidence to support the role of *P. shigelloides* as an enteric pathogen in man comes from case reports, studies of isolation rates from diarrhoeic and normal faeces, occasional case control studies and occasional volunteer studies. Some of this evidence is contradictory.

Extra intestinal infection is rare. There is little evidence to support the role of the bacterium in enteric disease in other animal species. Disease reports in cats (33,34,44) and the higher isolation rate in this species compared to other mammals (32) suggests that further studies in cats are warranted.

Drinking water (16,17,78), recreational water (23,80), and consumption of food contaminated with water (81) are the likely routes of infection in man. Case control studies support an association between disease and the consumption of fish, shellfish and contaminated water (60,63). Inadequate refrigeration and cooking probably contribute to infection (22). The routes of infection in animals are not known.

Enterotoxins, cytolysins, haemolysins and elastin are all produced by *P. shigelloides* and may play a role in the pathogenesis of disease. Plasmids probably do not play a significant role in infection (86) and there is no evidence of plasmid mediated antibiotic resistance (9).

Isolates of *P. shigelloides* are sensitive to cephalosporins, potentiated sulphonamides and quinolones in vitro. Resistance to penicillins is probably due to b lactamase activity and can be overcome with β-lactamase inhibitors (108).

A unified serotyping scheme exists incorporating over 100 somatic antigens and over 50 flagellar antigens. Serotype O:17 shares a common somatic antigen with *Shigella sonnei* and use has been made of this in preparing candidate vaccines against shigellosis (117,118).

P. shigelloides is not fastidious and grows in a range of enrichment broths and plating media. Veterinary, medical and food microbiology laboratories should all be encouraged to make frequent use of oxidase tests when sorting isolates in order to identify the bacterium and to obtain a more accurate picture of its prevalence.

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