tative tube agglutination using a working dilution of H-antisera. The H-agglutination of *Aeromonas* and *Plesiomonas* culture is flocculent in nature but it may only appear after incubation at 50 °C for 4 h or more.

Extrageneric relationships of mesophilic Aeromonas and Plesiomonas

Since *P. shigelloides* was first noted by Ferguson and Henderson[3] because of O-antigenic identity of their isolates with *Shigella sonnei*, additional antigenic relationships have been recognized between *Plesiomonas* and certain serovars of *S. dysenteriae* and *S. boydii*, as shown in table 1. Furthermore, many close O-antigenic relationships are also recognized among *A. hydrophila*, *P. shigelloides*, *Vibrio cholerae* and *Vibrio fluvialis*. Those are summarized in table 2. Whang et al.[10] reported the presence of the enterobacterial common antigen in strains of *P. shigelloides*.

Further problems with serotyping systems

Mesophilic *Aeromonas* includes three species: *A. hydrophila*, *A. sobria* and *A. caviae*. Since O- and H-antigens of the latter 2 species can be determined with antisera prepared against *A. hydrophila*, serovars of these species are included in a single scheme. Recently, Aldova[1] also published her own antigenic scheme of *Plesiomonas*, independently of Shimada and Sakazaki[2, 3], and includes 30 O-groups. Table 3 shows the relationships betwen the two schemes. It is desirable that one species have a single antigenic scheme which is used internationally. That aim will be achieved in collaboration between Aldova and Shimada and Sakazaki in the near future.

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II. Non-gastrointestinal diseases

Fish-pathogenic aeromonads, with emphasis on the ecology of *Aeromonas salmonicida*

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Key words. Aeromonas salmonicida; Aeromonas hydrophila; fish pathogens; aeromonads; uptake into fish.

Aeromonads, particularly *Aeromonas salmonicida* and to a lesser extent *A. hydrophila*, are recognised to be a scourge of fresh water fish farming worldwide[3]. *A. salmonicida*, which is the causal agent of furunculosis in salmonids, was initially recognised in the last century[6, 15] and has emerged as the best studied of all the bacterial fish pathogens. The significance of this pathogen has overshadowed *A. hydrophila*, which has been described as causing haemorrhagic septicaemia in a diverse array of fish species, including ayu, bass, carp, catfish, perch and salmonids[3].

Aeromonas salmonicida. Although Emmerich and Weibel[6] have been credited with the first description of furunculosis and the classification of the aetiological agent as *'Bacterium salmonicida'*, recognition should also go to Zimmermann[15], who named *'Bacillus devorans'*, a synonym of *'Bacterium salmonicida'*. An identical organism was described independently by Marsh[12] and classified as *'Bacterium truttae'*. Against this confusion, the pathogen was re-classified again, as *Aeromonas salmonicida*[7].

Ecology of Aeromonas salmonicida. By definition, *A. salmonicida* occurs only in fish but not in surface waters. Indeed, microbiological surveys of fresh water, even during the height of furunculosis epidemics, would seem to support this contention[1, 5]. However, such negative data do not explain the reasons for the spread of furunculosis between segregated fish populations or indicate the precise route of infection. Certainly, carrier or subclinically diseased fish may be partially responsible for transmitting the pathogen over small distances[13]. Yet, it seems unwise to neglect exploring fully the possibility of water-borne transmission. Consequently, against this background, work has been carried out to examine aspects of the survival of *A. salmonicida* in fresh water.

A detailed survey of two freshwater sites in England, using selective and non-selective media for aeromonads, failed to reveal the presence of *A. salmonicida* even from the water and sediments around populations of fish which were succumbing to furunculosis[1]. Nevertheless, the use of fluorescent antibody techniques (FAT) incorporating polyclonal antibodies to *A. salmonicida*, revealed the presence of the pathogen in aquatic samples during the height of furunculosis epidemics in July and August. Thus during these periods, micro-colonies of 5–10 coccoid cells, each of 1 μm in length/diameter, were observed on the surface of some water-borne particulates[2]. This suggested a total population of approximately 10^3 *A. salmonicida* cells/ml of fresh water. The viability of the fluorescing cells, which were attached to particulates, was confirmed using nalidixic acid and brain heart infusion broth (BHIB), in a procedure described by Kogure et al.[9]. Here, such coccoid cells enlarged to 2–3 μm in length within 6 h at 18 °C. Unfortunately, it was difficult to discern the exact nature of these particulates, but it was considered possible that they represented pieces of organic material, such as faecal matter or leaf debris. From September to June, there was a marked reduction in the number of *A. salmonicida* cells observed in the water column by FAT. At most, an occasional fluorescing coccoid bacterial-like cell was observed attached to particulates. Such data indicated a population density of ≤ 1 cell/ml. It is relevant to note that from laboratory-based experiments, Sakai[14] correlated the long term survival of virulent cells of *A. salmonicida* with their attachment to particulates, notably sand grains. However, it is impossible to relate such data to seasonal population trends in the natural environment.

From laboratory-based experiments, it was demonstrated that *A. salmonicida* could indeed survive in fresh water, albeit in an

apparent nonculturable or dormant state[2]. Although the inability to culture the pathogen from outwith fish may to some extent reflect an incomplete knowledge of subtle nutritional requirements, it should be emphasised that such a phenomenon could explain the explosive outbreaks of furunculosis in farmed salmonids, which have not experienced previous contact with diseased fish[11]. Moreover from laboratory-based experiments, it has been demonstrated that salmonids may indeed succumb to furunculosis following exposure to aqueous suspensions of the pathogen[4,8,11]. In a series of experiments using virulent and non-virulent cultures of *A. salmonicida,* which were grown in nutrient-rich (3.7% w/v BHIB) and nutrient-limited conditions (0.1% w/v BHIB), it was demonstrated that uptake of the pathogen into fish, i.e. rainbow trout, occurred within 2 min[8]. Thus, colonies of *A. salmonicida* developed on a selective isolation medium, peptone beef extract glycogen agar[10], following inoculation of tissue homogenates and blood with incubation at 22 °C for 48 h. Generally, the data revealed that low numbers, i.e. 2–25 cells of *A. salmonicida,* could be detected in the blood, kidney and spleen of the rainbow trout within 2-min exposure to the pathogen. There was negligible difference in the uptake of virulent or nonvirulent isolates. However, there was better uptake of cells derived from 0.1% (w/v) BHIB and in the presence of particulates, namely latex particles, compared to the other combinations. Superior uptake occurred by immersing the entire fish rather than just the head or tail regions. In all cases, the bacterial cells could not be detected in the fish after 24 h, and for that matter, furunculosis did not develop[8].

By examination of parallel samples by FAT, the presence of large numbers of bacteria in close contact with gill epithelial cells were observed within the 2-min exposure period. However, this is not surprising in view of the anticipated close contact between gills and bacterial suspension. Nevertheless, there was no evidence to indicate uptake of *A. salmonicida* across the gill epithelia, insofar as the bacteria were not observed in epithelial cells or within the capillaries. *A. salmonicida* was also observed as isolated cells on the lining of the lower intestine and rectum within 1 h after exposure to the bacterial suspension. Interestingly, such bacterial cells were not observed 3 h later, either indicating uptake into or elimination from the fish. There was no firm evidence for transport of *A. salmonicida* across the gut wall. Yet, this possibility was worthy of further investigation.

Certainly studies, to date, point to the presence of *A. salmonicida* on particulates in the aquatic environment, outwith of salmonids. It must be assumed that these cells comprise potential foci for infection of fish when certain, as yet unknown, conditions prevail. Such factors are likely to include the physiological state of the bacterial cells, which will be influenced by water temperature and the availability of specific nutrients, as well as the presence of appropriately 'stressed' fish. Then, uptake of the bacteria into fish occurs with considerable speed, indicating active rather than passive processes.

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Extraintestinal *Aeromonas* and *Plesiomonas* infections of humans

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Key words. Aeromonas; Plesiomonas; sepsis; meningitis; cutaneous infections; ecthyma gangrenosum; myositis; osteomyelitis; suppurative arthritis; pneumonia; peritonitis; cholecystitis; ocular infections.

Like other human enteric pathogens, *Aeromonas* and *Plesiomonas* can cause infections at sites outside the gastrointestinal tract[3,6]. Although these infections are uncommon, they tend to be severe and often fatal, particularly in individuals with impaired immunity. Based on published case reports, extraintestinal *Aeromonas* disease appears to be more frequent and varied than that due to *Plesiomonas* (table). Infections occur worldwide and patients of all ages have been described.

Sepsis. About 200 instances of *Aeromonas* bacteremia or sepsis have been recorded in the literature but clinical data are not available for all cases. Children and adolescents account for at least one-fourth of all patients. The majority have chronic underlying disorders including leukemia, solid tumors, aplastic anemia, hemoglobinopathies, cirrhosis, or renal failure. Patients with malignancies who develop *Aeromonas* sepsis are typically in relapse or have never achieved remission. The mortality rate exceeds 50% in spite of early treatment with antibiotics to which *Aeromonas* is susceptible in vitro[4,7]. Sepsis is frequently accompanied by infections of other sites such as the skin or lungs. When properly speciated, most blood isolates are found to be either *A. hydrophila* or *A. sobria*[9]. The source of *Aeromonas* is assumed to be the gastrointestinal tract although this organism has only infrequently been isolated from bacteremic patients from whom stool cultures were obtained.

Nine patients with *Plesiomonas shigelloides* sepsis have been described[3,8,10]. Five were neonates and the rest had underlying disorders such as sickle cell anemia, liver disease, cardiomyopathy, or rheumatoid arthritis. Eight had other foci of infection such as cellulitis, pneumonia, arthritis, or meningitis and the ninth was a neonate with mixed *Aeromonas* and *Plesiomonas* sepsis. Seven (78%) patients died.

Meningitis. Seven cases of *Aeromonas* meningitis have been reported to date[1,5,6]. They ranged in age from 13 days to 37 years. Underlying conditions included hemoglobinopathies, leukemia,