THE EPIDEMIOLOGY OF

SALMONELLA SEROVARS IN TASMANIA

BY

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This thesis contains no material which has been accepted for the award of any other higher degree or graduate diploma in any tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

Andrew Ball

ABSTRACT

The incidence of human salmonellosis and the distribution of *Salmonella* serovars in Tasmania was investigated to provide epidemiological information on *Salmonella* occurrence in Tasmania. This baseline data was not available previously and could contribute information to Australia-wide surveys of *Salmonella* and related enteric pathogens and, more specifically, aid in the prevention of enteric diseases in Tasmania. In addition, the relationship between *Salmonella* and indicator organisms in drinking water was investigated.

The incidence of human salmonellosis in Tasmania is 2% in patients with diarrhoea and 0.13% of the general (asymptomatic) population. This is comparable to rates on the Australian mainland and in other developed countries. The disease is most prevalent in summer which is also similar to seasonal distribution patterns elsewhere. However, the distribution of salmonellae in Tasmania is unusual in the prevalence of *Salmonella mississippi*, a serovar rarely encountered in mainland Australia. This serovar is not particularly invasive and is unremarkable in the age and sex distribution of its human hosts or its seasonal variation.

Epidemiological investigation of foods, domestic and wild animals, reticulated and natural waters, sea water and effluent were undertaken. No particular food type was implicated as a major source of *Salmonella mississippi*. Domestic animals, while having many *Salmonella* serovars in common with those seen in the human population, are not a significant source of *S.mississippi*. Human infection is likely to be frequently water-borne as 1.6% of 500 reticulated drinking waters and 8.2% of 250 natural fresh waters contained *Salmonella* of which 53% were *S.mississippi*. The peak incidence of *Salmonella* in water occurs in early summer and precedes the summer maximum of human cases.

Salmonella mississippi was isolated from several species of carnivorous and insectivorous mammals and reptiles but not herbivores. Fifty percent of 120 native cats (*Dasyurus viverrinus*) sampled were infected with *Salmonella* and *S.mississippi* comprised 97% of these. There was no apparent seasonal variation of the presence of *S.mississippi* in native cats. This serovar persisted in native cats for at least 2-3 months while on a *Salmonella*-free diet. During this time no symptoms or ill effects were apparent.

To establish how native cats became infected, components of their diet were tested. *Salmonella mississippi* was not isolated from the common pasture pests which compose the bulk of their diet. However, it was isolated from 62% of 34 metallic skinks (*Niveoscincus metallicus*) sampled on which they sometimes prey. This is the commonest of 16 Tasmanian skink species and is endemic to Tasmania and south east Victoria.

The reservoir of *Salmonella mississippi* in Tasmania appears to be the native animal population which contaminates water supplies leading to sporadic human infections.

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INTRODUCTION

In Tasmania, the coordination and investigation of notifiable diseases has been poor. Notifications were used to compile annual statistics only. The recognition of disease outbreaks has occurred too late in most cases, to investigate them effectively. As a consequence, the epidemiology of enteric diseases is largely unknown.

Little was known about the epidemiology of *Salmonella* in Tasmania or whether it follows the same demographic distribution as mainland Australia. It has been noted that one serovar, *S.mississippi*, seems to be more prevalent in Tasmania than elsewhere. The sources of human salmonellosis have rarely been sought and little information is available about veterinary salmonellosis. The contribution of food, water, animals or pollution to the occurrence of *Salmonella* in Tasmania has not been investigated.

The primary aim of this work was to investigate the epidemiology of salmonellae in Tasmania, particularly *S.mississippi*, and to improve the effectiveness of enteric disease notifications and subsequent investigations. In addition, preliminary studies were carried out on factors affecting the growth and survival of *Salmonella mississippi* and other *Salmonella* serovars to indicate characteristics of the former that might provide an explanation for its persistence in Tasmanian environment.

LITERATURE REVIEW

NOTIFIED FOOD POISONING AND ENTERIC DISEASES IN TASMANIA

The incidence of food-borne and enteric bacterial diseases in Tasmania and mainland Australia are compared in Table 1. It is apparent that *Salmonella* is an important gastro-intestinal pathogen in both Tasmania and Australia. Salmonellosis is six times more prevalent than shigellosis in Australia whereas shigellosis is relatively insignificant in Tasmania where *Salmonella* cases outnumber *Shigella* thirty-fold. Incidence of *Vibrio* and enteropathogenic *E.coli* (EPEC) gastroenteritis is not commonly reported throughout Australia. Figures for *Campylobacter* are not available because it was not notifiable in some states. However, during the past 12 months, since *Campylobacter* was added to the list of notifiable diseases in Tasmania, a case rate of approximately $100/10^5$ was reported in northern Tasmania during 1990 (Dr.L.Lyons, Department of Health, unpublished data). A figure covering the entire state was not possible due to inadequate reporting from Hobart.

TABLE 1. NOTIFIED ENTERIC DISEASES IN THE PERIOD 1985 - 1989

PATHOGEN	CASE RATES PER 100,000 AUSTRALIA	TASMANIA
Salmonella	31	25
Shigella	4.7	0.8
Vibrio	0.02	0
EPEC	0.14	0

The significance of such diseases has been investigated in the USA (Bean & Griffin, 1990), the estimated cost to the economy being US$8,000,000,000 per annum. *Salmonella* comprised 45% of the bacterial pathogens reported, and accounted for two thirds of the food poisoning cases or 2.3 billion dollars. If similar conditions occurred in Tasmania the estimated annual cost to the local economy would be in the order of $5,000,000. This indicates that identification of common sources of *Salmonella* and reduction in human salmonellosis in Tasmania could result in significant cost saving and benefit to the community.

Examination of *Salmonella* Reference Laboratory annual reports (IMVS, 1980-89) revealed that certain serovars predominate in Tasmania. That

S.typhimurium is almost invariably the most common serovar is expected as it predominates in most surveys world-wide (Van Oye, 1964; Christopher *et al.*, 1974). However, the next most frequently reported serovar is *S.mississippi*, which is rarely reported in other states. *Salmonella mississippi* was the predominant human serovar in Tasmania in several years (1982, 1988 and 1990) which is most unusual.

Salmonella mississippi was first described in 1943 and was isolated from a stool specimen from a food handler in Florida, USA. A world-wide survey of all *Salmonella* serovars was published in 1964 (Van Oye, 1964) which showed *S.mississippi* to be a very rare serovar. Since the original report it had been reported on only ten occasions at the locations shown in Table 2. The first Australian case of *S.mississippi* occurred in an army camp in Northern Australia during the Second World War (Atkinson, 1964). It is not clear whether Australian or American personnel were involved.

TABLE 2. WORLDWIDE INCIDENCE OF *SALMONELLA MISSISSIPPI* PRIOR TO 1964

LOCATION	SPECIMEN	YEAR ISOLATED
USA	1 human	1943
UK	2 human	1956, 1962
	1 coconut	1953
South Africa	1 egg	1960
Canada	1 human	1956
Ecuador	1 human	
Central Africa	1 human	
Australia	2 human	1944
	1 other	1945

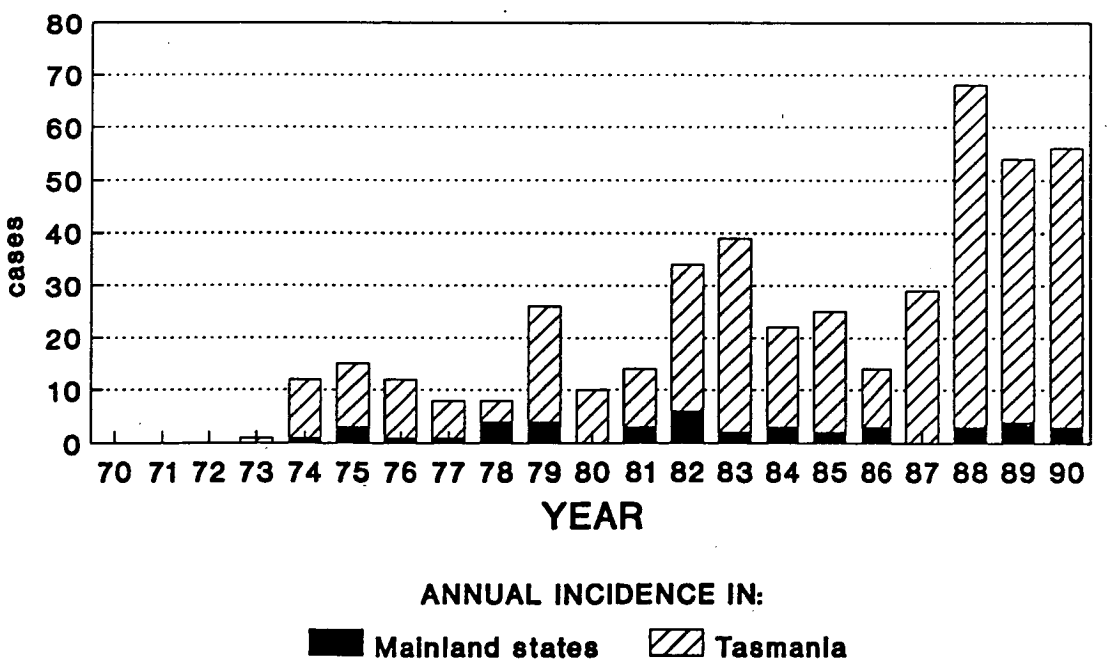
Human surveys are a more reliable guide to the trends in salmonellae than non-human surveys. Other surveys, carried out for a variety of reasons, tend to introduce bias into quantitative results. For this reason isolations of *S.mississippi* from human sources were used to compare frequency of occurrence with time or geographic location.

Since 1970 *Salmonella mississippi* was more frequently isolated, particularly in the USA, Australia and New Zealand. Most Australian isolations were localised in Tasmania. The occurrence of *S.mississippi* in Tasmania and mainland Australia is shown in Figure 1. In the period between 1980 and 1989 *S.mississippi* comprised between 18 and 62 percent of salmonellae isolated from humans in Tasmania compared with between 0.060% and 0.697% for the remainder of Australia.

In the USA, during 1989, *S.mississippi* isolations were focussed in certain states within the southern regions (CDC, 1989). Specifically these were; Georgia, South Carolina, Alabama, Tennessee, North Carolina, Texas and Louisiana. The proportion of *S.mississippi* among human salmonellae in the USA during 1979-1989 ranged from 0.23% to 0.44% with a mean of 0.34%. This compares with 1.76% during 1989 in the southern regions, where it is most prevalent, but clearly does not approach levels recorded in Tasmania.

It was estimated that more than half the cases of human salmonellosis, diagnosed by medical practitioners in Tasmania, were not confirmed by pathological testing (R.G.Tucker, Director of Microbiology, Royal Hobart Hospital. pers. comm.). Moreover it is widely regarded that many patients with *Salmonella* gastroenteritis did not consult a medical practitioner about the condition as it was usually self limiting (E.J.McArdle, Director of Public Health. pers. comm.). Therefore the incidence of *Salmonella* in Tasmania may be much higher than the statistics indicate, as observed in the USA where an estimated 1-3% of cases were reported to public health authorities (Chalker & Blaser, 1988, Dauer (1960) cited by Galton, 1966).

FIG. 1 ANNUAL INCIDENCE OF *S.MISSISSIPPI* IN AUSTRALIA



1970-79 data from IMVS, Adelaide
1980-90 data from NSSS

HUMAN SALMONELLOSIS

SYMPTOMS.

Salmonella can cause five different clinical conditions; gastroenteritis, bacteraemia, enteric fever, localised infections and asymptomatic carriage (Gorbach, 1983). However there is considerable overlap between these (Black *et al.*, 1960).

Gastroenteritis is the predominant syndrome, occurring in >70% of cases. The incubation period is normally 6-48 hours but may be up to 12 days. Symptoms are usually nausea and vomiting, followed by abdominal cramps, diarrhoea and often fever. The diarrhoea can vary in its severity and frequently lasts 3-4 days, although occasionally it may be intermittent for several weeks. Diarrhoea is caused by *Salmonella* invading the intestinal epithelium, particularly the distal ileum and to a lesser extent, the colon. Non-invasive strains fail to produce diarrhoea (Giannella *et al*, (1973) cited by Gorbach, 1983).

Bacteraemia occurs in about 10% of cases and is signified by persistent fever with or without gastrointestinal symptoms.

Enteric fever is characterised by the typical typhoid symptoms of prolonged chills, fever, headache, nausea, vomiting, enlargement of the spleen, and "grape skin" stools. It is characteristic of all typhoid cases and approximately 8% of other *Salmonella* infections.

Once the bacterium invades the blood, localised infections of almost any organ can occur. Arteritis, endocarditis, meningitis, osteomyelitis, septic arthritis, wound infections, or focal abscesses are seen in about 5% of cases.

Chronic carriers occur at a rate of 0.2-0.6% as a consequence of either symptomatic or asymptomatic infection. Infants, the elderly, and patients with gall or kidney stones are the most likely members of the population to become asymptomatic carriers (Gorbach, 1983).

PREDISPOSING FACTORS.

There are a number of medical conditions and other predisposing factors which increase the risk of salmonellosis (Gorbach, 1983), and so may be relevant to case histories in epidemiological investigation. These include haemolytic anaemias, such as those caused by sickle cell disease, malaria, bartonellosis,

and louse-borne relapsing fever; neoplastic diseases, particularly leukaemia, lymphoma, and disseminated malignancy; gastric surgery; schistosomiasis; ulcerative colitis; immunosuppression due to pregnancy (Morse & Duncan, 1974) or drugs, and treatment with antibiotics. The incidence of osteomyelitis due to *Salmonella* is greater in patients with haemolytic anaemia (Hook, (1961) cited by Gorbach, 1983). Septicaemia is facilitated by neoplastic disease and exacerbated by treatment with chemotherapy and radiotherapy, along with other drugs which suppress the immune system. Gastric surgery, schistosomiasis, and ulcerative colitis all enhance the development of *Salmonella* infection.

Other factors may influence the course of infection and its severity. It is widely regarded that infants and the aged are more susceptible to salmonellosis (Sickenga, 1964; CDC, 1989). The cause of this apparent disparity has been postulated to be decreased stomach acidity (Blaser & Newman, 1982). Infants under two months of age produce little hydrochloric acid, and the elderly have a higher incidence of achlorhydria than the general population; both increase the stomach pH. The same mechanism may explain the increased susceptibility of patients taking antacids (Giannella *et al*, (1973) cited by Blaser & Newman, 1982). Rapid passage through the stomach may also allow a low dose to lead to salmonellosis. It has been shown that a small amount of water passes rapidly through an empty stomach (Mossel & Oei, 1975) which may explain cases of water-borne salmonellosis from lightly contaminated water. Another case which reduced the contact with stomach acidity involved an infected gastroscope (Joseph & Palmer, 1989). In outbreaks where very low numbers of the infective organism were found in chocolate (Craven *et al.*, 1975; Gill *et al.*, 1983) and cheese (Ratnam & March, 1986) it has been proposed the salmonellae were shielded from the stomach acid by fat. The general state of health and the natural (genetic) resistance to salmonellae may also be relevant (Sickenga, 1964). The dose, strain and serovar of the infecting organism also has a bearing on morbidity (Blaser & Newman, 1982).

The mortality of patients with non-typhoid salmonellosis is generally low in developed countries, 2-2.3% being reported in the UK (Abbott *et al.*, 1980). The mortality rate is higher in patients with other medical problems such as malignancy, chronic infections, arteriosclerosis, nephritis, diabetes, anaemia, and alcoholism (Sickenga, 1964). Higher mortality is also age dependent, as shown by UK surveys in which 77% of the patient mortalities occurred in over

60 year olds and 17% were infants of less than 3 months, with the remaining 6% between these extremes (Abbott *et al.*, 1980).

Infective Dose

Woodward (1983) (cited by Blaser & Newman, 1982) suggested the dose-response curve for typhoid infection is linear with respect to the logarithmic dose. If the same is valid for other salmonellae and low doses do cause infection in a smaller proportion of the population, the sporadic nature of salmonellosis may be explained. Some salmonellae are much more virulent than others. For example, 10^9-10^{10} *S.pullorum* were required to infect human volunteers compared with doses of 10^4-10^5 for various other serovars (McCullough & Eisele, (1951) cited by Sickenga, 1964).

Carriers

The importance of carriers in the spread of *Salmonella* has been noted by several workers (Palmer & Rowe, 1983; Kotova *et al.*, 1988). However, what is meant by the term "carrier" differs. Patients who excrete the organism for a variable period of time and eventually rid themselves of it will be referred to as excretors. Others seem to become perpetual, if intermittent, excretors or chronic carriers. There is probably a very fine line between the two. Chronic typhoid carriers are associated with gallstones (Christie, 1974). Affinity of other serovars to gallstones (Forbes & Cotton, 1984) and kidney stones (Sarmina & Resnick, 1986) suggest this mechanism may apply generally to chronic carriers. Antibiotic treatment is known to extend the excretion period in *Salmonella* infections (Dixon, 1965) but has no effect on the carrier state (Szmuness, 1966).

The proportion of patients excreting is generally regarded to decrease logarithmically with time. Various aspects affecting this phenomenon have been reviewed (Buchwald & Blaser, 1984). Age and sex are the most important determinants in the development of the carrier state (Black *et al.*, 1960), the elderly and very young having the highest carriage rates. In *S.typhi* cases 10% of patients over 60 years old become chronic carriers compared with 0.3% of the under 20 year old age group (Ames & Robins, 1943). The ratio of female to male carriers is about 2:1 for both typhoid and non-typhi salmonellae. The clinical status of long term excretors may have an effect in that symptomatic excretion appears to be more persistent than asymptomatic, although other

factors may have a bearing on this. The strain of the infecting *Salmonella* may also be important. *S.typhi* is known to persist in a higher percentage of excretors for longer (Christie, 1974). Recovery from *S.typhimurium* infection appears to be faster than other non-typhi strains but the proportion of permanent carriers are approximately equal (Buchwald & Blaser, 1984). The incidence of chronic carriage of non-typhi *Salmonella* is, however, rare (Black *et al.*, 1960). Temporary carriers are more frequent and probably maintained by the organism multiplying in the gut and becoming a transient member of the commensal microbiota (Black *et al.*, 1960). Extended excretion may involve the gall bladder, mesenteric lymph nodes, intestinal diverticula or anomalies of the urinary tract (Sickenga, 1964).

The concentration of *Salmonella* in faeces also decreases during recovery (Pether & Scott, 1982). Children tend to excrete higher levels for longer than adults, especially before an adult diet is initiated (Pether & Scott, 1982). Faecal levels of 10^6-10^7 from children during early convalescence are commonly encountered for weeks to months compared with adults who excrete much lower levels within a fortnight of clinical recovery (Cruickshank & Humphrey, 1987). Stools from short term and symptomatic patients generally contain more *Salmonella* than those of long term and asymptomatic excretors respectively (McCall *et al.*, 1966).

The numbers of *Salmonella* occurring in diarrhoea is very variable. In patients with active diarrhoea, levels between 10^5 and 10^9 per gram of faeces are normal compared with 10^4 - 10^8/g for asymptomatic excretors (Donaldson & Toskes, 1983). However levels of 1,000 - 10,000/g and 10 - 100/g have been reported in one third and one fifth of diarrhoea specimens respectively. In general salmonellosis causes a decrease in the numbers of normal enteric bacteria and an increase in the proportion of salmonellae, which become more visible on direct plates of the appropriate agars.

INCIDENCE.

A number of human *Salmonella* surveys have been reported in the literature. Surveys of the general population (Cruickshank & Humphrey, 1987) show incidence rates of 0.2-5% in the USA and 0.15% in Japan. A similar incidence of 0.15% was found in children under the age of 5 years in the UK. Higher levels have been reported in less developed countries. A survey of healthy and sick villagers in Panama yielded a 9.1% isolation rate (Kourany & Telford,

1981). In Sierra Leone a survey of villagers tested at random resulted in a 6% isolation rate (Wright, 1982).

Outbreaks

Investigations of outbreaks are useful in indicating the proportion of asymptomatic excretors in particular circumstances. In a survey of outbreaks in hospitals in the UK 3% of patients and 5% of staff were found to be excreting *Salmonella* without showing symptoms (Palmer & Rowe, 1983). In two of these outbreaks the incidence in catering staff was highlighted with 6.8 and 16% asymptomatic excretors detected. Similarly, of hospitalised children under 2 years old in the USSR, between 2 and 7% were chronic, intermittent excretors (Kotova *et al.*, 1988).

SALMONELLA IN ANIMALS

Pathology

Salmonellae are widely distributed in the animal kingdom. Some serovars display host specificity and cause defined symptoms in their host (Table 3) but are found less commonly and cause non-specific salmonellosis in other animals (Savage *et al.*, 1940; Buxton, 1957; Sickenga, 1964; Prost & Riemann, 1967).

TABLE 3. Host-specific *Salmonella* serovars

SEROVAR	HOST	DISEASE
S.typhi	Human	Typhoid fever
S.paratyphi B	Human	Enteric fever
S.abortus equi	Horse	Abortion
S.abortus ovis	Sheep	Abortion
S.choleraesuis	Pigs	Swine fever
S.pullorum	Poultry	Bacillary white diarrhoea
S.gallinarum	Poultry	Fowl typhoid

The remaining serovars, which are termed non-specific *Salmonella*, are usually not host associated but some serovars are commonly found in certain animal species: *S.dublin* and *S.typhimurium* with cattle (Buxton, 1957; Prost & Riemann, 1967); *S.oranienberg, S.typhimurium, S.dublin* and *S.java* with sheep (Prost & Riemann, 1967); *S.choleraesuis* and *S.typhimurium* with pigs (Prost &

Riemann, 1967); *S.typhimurium*, *S.enteritidis* and *S.anatum* with ducks (Galton, 1966); and *S.typhimurium* with horses (Carter *et al.*, 1986). Their presence in animals is due to one of several reasons:

Primary salmonellosis - where *Salmonella* is the cause of the disease.
Secondary salmonellosis - where other diseases or stress may weaken the animal and allow a latent infection to become more generalised and develop into a secondary *Salmonella* infection.
Asymptomatic salmonellosis - where *Salmonella* is present in clinically healthy animals. Salmonellae are most commonly found in the gastrointestinal tract and mesenteric lymph nodes of asymptomatic animals (Prost & Riemann, 1967).
Mechanical carriers - where the salmonellae are consumed by the animal but no infection occurs. The *Salmonella* exists in the animal just long enough to pass through the alimentary system and be excreted (Fenlon, 1981).

Clinical Manifestations

There has been much investigation of salmonellosis in farm animals as they are recognised as a major source of human food-borne salmonellosis. Domestic animals have been investigated to a lesser extent as they are a source of human zoonotic infection. Wild animals are seldom regarded as a significant cause of human salmonellosis. Symptoms and outcomes of salmonellosis vary between host species and are discussed below to recognise diseased animals.

Cattle.

Clinical salmonellosis in cattle has been reported to be most common in young calves and uncommon in adult cattle (Rokey & Erling, 1959; Rothenbacher, 1965). The high incidence in calves may be due to intensive methods of animal husbandry, such as communal feeding practices or differing rumen conditions in adult cattle and young calves (Buxton, 1957). The converse has been noted in New Zealand (Salisbury, 1958) where less intensive farming is generally practiced. Symptoms are sudden onset, acute scouring, loss of milk and condition, elevated temperature and intense thirst. The mortality rate in calves can be as high as 33% (Rokey & Erling, 1959; Rothenbacher, 1965) and death occurs usually within 24-48 hours (Salisbury, 1958).

Sheep.

Ovine gastroenteritis manifests itself as a light coloured, persistent and often bloody diarrhoea in sheep which are dull, not feeding and may have arched backs and complete wool break (Watts & Wall, 1952; Robinson, 1970).

Mortality of 66% has been reported in lambs involved in outbreaks of salmonellosis (Prost & Riemann, 1967).

Pigs.

Pigs can show three salmonellosis syndromes (Salisbury, 1958), pigs less than six months old being particularly susceptible. In preacute disease, or "blue belly", death occurs within 48 hours of a loss of appetite and increased respiration. The pigs are often found dead without first appearing sick. Acute salmonellosis manifests itself as a prolonged loss of condition often with diarrhoea and pneumonia and sometimes lameness. The presence of an increased number of runts and slow growing piglets is a sign of the chronic syndrome. This usually occurs as the aftermath of a previous acute attack.

Horses.

Three clinical forms are observed (Morse *et al.*, 1976).

Periacute - toxaemia or septicaemia of rapid onset and often fatal; frequently seen in foals. Symptoms are weakness, depression, anorexia and elevated temperature (>40°C).

Acute - fever, weakness, anorexia and diarrhoea containing blood and mucus.

Chronic - weight loss, dehydration, diarrhoea ("cow pat" faeces).

S.typhimurium appears to be more lethal with a 60% mortality rate, approximately double that of other salmonellae (Carter *et al.*, 1986).

Dogs.

Clinical symptoms of early fever are followed by diarrhoea of varying severity and sometimes signs of respiratory infection (Galton *et al.*, 1952) or dehydration, prostration, bacteraemia, anaemia and icterus (Venter, 1988).

Cats.

Salmonellosis in cats is usually characterised by diarrhoea and sometimes vomiting, fever and conjunctivitis (Dow *et al.*, 1989), and has high morbidity and mortality (Galton, 1966).

Poultry

Depression, drooping of wings, hyperexcitability, transient diarrhoea, liver lesions, peicarditis, paresis and growth depression are symptoms seen in poultry (Awaad *et al*, 1981, Hungerford, 1969).

Fish.

After a massive feeding dose of *Salmonella*, pseudomembranous inflammation

of the gut may be observed at *post mortem* examination (Heuschmann-Brunner, 1974).

Invertebrates

Cockroaches - no obvious changes but increased mortality (Mackerras & Pope, 1948).

Fleas - dark, cloudy appearance of the stomach after a meal of blood, rather than the normal uniform bright red. Sometimes bloody diarrhoea with increased mortality (Kramer, 1963).

Ticks - swelling and dark brown colouring of the body and reddening of the legs due to diffusion of ingested blood. Increased mortality (Kramer, 1963).

PATHOGENESIS (MODE OF INFECTION, INFECTIVE DOSE)

While ingestion is probably the most important mode of infection, several other routes exist. The respiratory route was demonstrated in mice (Darlow *et al.*, 1961; Tannock & Smith, 1972). Entry via the conjunctiva has been shown in guinea pigs (Moore, 1957; Belfort *et al.*, 1985), and mice (Trillat & Kaneko (1921) cited by Darlow *et al.*, 1961). Direct inoculation into the blood by ticks and fleas has been suggested (Kramer, 1963). Transovarian infection of bird and reptile eggs with *Salmonella* has been documented (Awaad *et al.*, 1981; Izadjoo *et al.*, 1987; Humphrey *et al.*, 1989). The anus has been implicated as a portal of entry to the intestine in humans via contaminated rectal thermometers (Im *et al.*, 1981), and turtles by cloacal uptake (Izadjoo *et al.*, 1987).

The concept of infective dose is rather nebulous and it is questionable whether it is of much value except in clinical trials where all variables can be strictly regulated. Apart from host and serovar variations, the condition of the animals is important (Hart *et al.*, 1985). Feeding also has a bearing; the infective dose in sheep, normally 10^7-10^8, can be reduced to 10^2-10^3 after fasting (Grau & Brownlie, 1965). Variation between members of the same host species may account for some differences in infectivity, as shown in one flock of chickens where the percentage infection was proportional to the magnitude of the dose (Gustafson & Kobland, 1984). The site of entry to the host has perhaps most significance on the infective dose. An oral dose may be several orders of magnitude higher than that of other routes (Moore, 1957; Darlow *et al.*, 1961; Gibson, 1965) such as respiratory or conjunctival.

The course of infection varies between host species. It is influenced by the type and virulence of the *Salmonella*, the dose and portal of entry, and by host factors such as stress and concurrent diseases.

Stressed animals are more susceptible to disease. This has been shown in cattle (Salisbury, 1958; Frost *et al.*, 1988), horses (Morse *et al.*, 1976), poultry (Popiel & Turnbull, 1985), mink (Gorham *et al.*, 1949), mice (Tannock & Smith, 1972), sheep (Salisbury, 1958; Tannock *et al.*, 1971), macropods (Samuel, 1981), lizards (Lee & Mackerras, 1955) and quokkas (Hart *et al.*, 1985).

Salmonellosis has reportedly been induced by many forms of stress; starvation (Gibson, 1965; Williams & Bellhouse, 1974; Hart *et al.*, 1985; Popiel & Turnbull, 1985), concurrent infections (Gibson, 1965; Morse *et al.*, 1976), heat (Hart *et al.*, 1985), nutritional deficiencies (Gibson, 1965; Hart *et al.*, 1985), post-calving (Salisbury, 1958), transportation (Ryan, 1972), overcrowding (Taylor & McCoy, 1969), post-surgery (Morse *et al.*, 1976), after treatment with antibiotics (Morse *et al.*, 1976; Sun, 1984) or dietary change (Frost *et al.*, 1988).

INCIDENCE (SURVEYS, SEASONAL, AGE, GEOGRAPHICAL VARIATION)

There have been many *Salmonella* surveys of farmed animals, primarily because of their potential to infect humans. Of the surveys of healthy animals, in Australia, New Zealand, Europe and the USA, most show a fairly low incidence of *Salmonella*; 0.4 - 1% of cattle (Smith & Buxton, 1951; Taylor & McCoy, 1969), 0.4 - 2.5% of poultry (Smith & Buxton, 1951), 0.2 - 2.1% of horses (Smith & Buxton, 1951; Morse *et al.*, 1976; Begg *et al.*, 1988), 0.7% of pigs (Smith & Buxton, 1951), <0.2% of sheep (Smith & Buxton, 1951) and 3.9% of mink (Williams & Bellhouse, 1974).

There is significant variation of carriage rates between certain countries. Horses in South America, for example, are reported to carry salmonellae at rates between 17 and 27% (Morse *et al.*, 1976), which far exceeds that of more developed countries. In this case the high incidence may be due to poor levels of hygiene or possibly that the horses were not in good condition as they were to be used for food.

The source of animals can also be significant. The apparently high infection rate of 16% of horses in a Queensland survey becomes less significant when

the source is identified as hospitalised animals (Roberts & O'Boyle, 1981). Animals surveyed in such conditions are not truly representative of their species at any given time or place. Apart from the fact that sick animals may have salmonellosis, thus artificially increasing the proportion carrying *Salmonella* (Roberts & O'Boyle, 1981), close contact with infected animals can increase the chances of spread to healthy individuals. This was exemplified by a survey of healthy calves which increased from 0.6 to 36% salmonella carriers after yarding for up to five days (Anderson *et al.*, 1961). Similarly, holding pigs in pens for prolonged periods increased the incidence of *Salmonella* within the herd (Hanson *et al.*, 1964).

Domestic pets have also been closely scrutinised as potential sources of zoonotic salmonellosis. The incidence of dogs carrying salmonellae ranges from 0.5 to 26% with 6.9% in Australia (Galton *et al.*, 1952; Frost *et al.*, 1969; Morse & Duncan, 1974; Borland, 1975); cats between 0.5 and 12% (Cruickshank & Williams Smith, 1949; Morse & Duncan, 1974; Borland, 1975);and tortoises, 12 - 85% (Speare & Thomas, 1988). Australian surveys of domestic animals show results which are more similar to those of north America and Europe than elsewhere.

There have been many surveys of wild animals, usually to ascertain their potential risk to the human or domestic animal population.

Rodents, which are traditionally regarded as major disseminators of disease, have undergone close scrutiny. The incidence of *Salmonella* in rats varies between 0.3 and 7.3% (Cruickshank & Williams Smith, 1949; Brown & Parker, 1957; Schnurrenberger *et al.*, 1968; Williams, 1975; Singh *et al.*, 1980) and between 0.3 and 10% in mice (Cruickshank & Williams Smith, 1949; Brown & Parker, 1957; Tannock *et al.*, 1971; Jones & Twigg, 1976; Singh *et al.*, 1980).

Birds which commonly frequent drinking water supplies have also been examined. Various surveys have shown 4-16% of wild ducks (Muller, 1965; Mitchell & Ridgwell, 1971) and 2.9-78% of gulls (Muller, 1965; Coulson *et al.*, 1983; Fricker, 1984; Monaghan *et al.*, 1985) to carry *Salmonella*. The highest isolation rates were from birds which were known to feed on sewage or at rubbish tips (Butterfield *et al.*, 1983; Fenlon, 1983) and may not be representative of all such wild fowl. Pigeons, which live in close association with man in urban environments, also showed high carriage rates of between 6 and 30% (Muller, 1965; Mitchell & Ridgwell, 1971). Other birds surveyed

generally displayed much lower levels, often less than 1% (Muller, 1965; Goodchild & Tucker, 1968; Schnurrenberger *et al.*, 1968; Plant, 1978).

Surveys of *Salmonella* in other wild animals have shown variable results- opossums, 1.3-22% (Schnurrenberger *et al.*, 1968; Marx, 1969); feral cats, 3.8-15% (Schnurrenberger *et al.*, 1968; Marx, 1969); raccoons, <1-17% (Marx, 1969; Bigler *et al.*, 1974); foxes, 1.2-7% (Marx, 1969); shrews, 1-11% (Jones & Twigg, 1976; Singh *et al.*, 1980); frogs, 14-40% (Sharma *et al.*, 1974; Sarvamangala & Shivananda, 1983) and quokkas, 0-100% (Hart *et al.*, 1985). Such variation seems to be a function of environmental factors rather than the species. However reptiles seem to harbour salmonellae most frequently with isolation rates reported between 23 and 77% for lizards and 78-92% for snakes (Iveson *et al.*, 1969; Iveson & Hart, 1983; Gugnani *et al.*, 1986). A Dutch survey reported an isolation rate of 95% for a mixed sample of various reptiles (Koopman & Janssen, 1973).

EPIDEMIOLOGY

The epidemiology of *Salmonella* is a highly complex web of interrelationships between man, animals, their food and the environment.

Humans are major disseminators of *Salmonella* to the environment but also to food, animals and other humans. Commonly used methods of human waste disposal may contribute significantly to environmental pollution as approximately 0.15% of the population excrete between 10^2 and 10^5 salmonellae per gram of faeces (McCall *et al.*, 1966; Cruickshank & Humphrey, 1987). The concentration and number of different serovars present in sewage varies depending on the type of sewage treatment (Jones *et al.*, 1980; Fenlon, 1983). The presence of salmonellae in sewage is commonplace (Harvey *et al.*, 1969; Linklater *et al.*, 1985) and tends to reflect the active and latent infections in the community. Levels as high as 5,000 *Salmonella* per litre have been reported (Muller, 1965). Excretors have even been identified by progressive testing of the contents of a sewerage system (Hobbs & Gilbert, 1978). Rubbish tips may also be a repository of salmonellae, and not only from the dumping of sanitary waste and septic tank effluent (in areas where it is allowed) but also from domestic meat waste (Durrant & Beatson, 1981).

The number of human infections caused by direct person to person spread is very low (McCullough & Eisele, (1951) cited by Sickenga, 1964; MacGregor &

Reinhart, 1973), although is more likely between mother and infant. This mode of transmission has been implicated in hospital outbreaks. Its occurrence in paediatric populations is not questioned but where adults are involved infection by other routes, especially food, have been reported to be more likely (MacGregor & Reinhart, 1973). However this finding has been questioned in epidemiological investigations of hospital outbreaks in the UK which laid the blame on poor ward hygiene rather than hospital catering (Palmer & Rowe, 1983). In some cases nursing and medical staff were found to be excreting the same serovar as patients with salmonellosis while the kitchen and catering staff were cleared.

Transmission from man to animals is thought to be very low but is not often investigated. An instance of salmonellosis in a family on a farm followed by a cow developing clinical symptoms has been described, (Messerli (1962) cited by Gibson, 1965), where *S.typhimurium*, which is more prevalent in man than cattle, was isolated from both sources.

Contamination of food by human carriers and excretors is widely documented (Felsenfeld & Young, 1949; Hobbs & Gilbert, 1978). Fingers inoculated with low levels of *Salmonella* were still contaminated after hand washing and up to three hours afterwards (Pether & Gilbert, 1971) and were effective in contaminating other food. In most instances, however, the source of the inoculum was thought to be contaminated food rather than excretors (Pether & Gilbert, 1971; Cruickshank & Humphrey, 1987). Whatever the original source of infection, poor hygiene practices were estimated to contribute to 63% of food-borne salmonellosis outbreaks investigated in the USA between 1973 and 1987 (Bean & Griffin, 1990).

Food is the most significant source of human salmonellosis. In a major epidemiological study undertaken in the USA over the 14 year period to 1987 *Salmonella* was found to be responsible for 28% of food poisoning outbreaks and 44% of cases (Bean & Griffin, 1990). This compares with 5.8% of outbreaks and 33% of cases in Canada during 1983-4 (Todd, 1989) and 23% of UK outbreaks in the 1973-5 period (Vernon (1977) cited by Hobbs & Gilbert, 1978). Meat, poultry and dairy foods are the most frequently implicated foods in these surveys of outbreaks but the case incidence was highly variable. This is because a few large outbreaks due to particular foods greatly affect the case statistics. The USA survey identified commercial and institutional eating places rather than home as the source of most food poisoning outbreaks and cases.

The major factors contributing to the cause of the outbreaks were improper holding temperatures for flesh foods, pre-cooked foods and bakery products; inadequate cooking for fruit and vegetables; and food from unsafe sources for shellfish and dairy foods. There are no such comprehensive Australian surveys.

A greater percentage of food handlers, especially those who handle raw meat, have been found to excrete *Salmonella* (Pether & Gilbert, 1971). This seems to be an occupational hazard of working in an environment rich in salmonellae, which can persist on the hands for several hours and increase the chances of infection.

Cross-contamination is the term used for one food contaminating a previously unsoiled one. It can be mediated by the food handler or the manufacturing process, or inadequate processing (Pether & Gilbert, 1971; Hobbs & Gilbert, 1978) and is exacerbated by inadequate conditions during storage and transport or by the use of heavily contaminated ingredients (Bean & Griffin, 1990).

Contaminated waste food is sometimes dumped on rubbish tips (Durrant & Beatson, 1981), where conditions may be conducive to the multiplication of the contaminant microorganisms and serve as a source of contamination to scavengers (Grunnet & Brest Nielson, 1969). Waste food may similarly be a source of *Salmonella* if it is used to feed domestic animals such as pigs but this practice is forbidden in Tasmania.

Domestic animals frequently harbour salmonellae and play a large part in their dissemination throughout the various pathways of the *Salmonella* cycle.

Domestic animals used for food are said to be a significant source of human food poisoning with some studies showing 69% of traceable human salmonellosis outbreaks from food animals (Holmberg *et al.*, 1984). Meat and poultry products caused 47% of successfully investigated outbreaks in the UK between 1949 and 1959 (Galbraith, 1961) and over 70% in the following ten year period (Lee, 1974).

Similarly animal products used as stock feeds have added to the incidence of both the numbers and serovars present (Lee, 1974). Cross contamination of the finished product with contaminated unprocessed material was regarded as the principal infection mechanism (Morehouse & Wedman, 1961).

The spread of infection between stock is also an important mode of dissemination. This has been shown to be particularly prevalent during

transport and at holding pens at abattoirs (Moule & Young, 1951; Anderson *et al.*, 1961; Hanson *et al.*, 1964). Under such circumstances carrier animals were particularly important (Josland, 1953) and a small number of excreting beasts can infect a significant proportion of the herd. It has been suggested that feed changes and stress due to transport are more important than prolonged penning (Frost *et al.*, 1988). This effect was shown when more salmonellae were isolated from cattle after 18 days in a feedlot than at the sale yard. None were isolated after 80 days on the feedlot. Infected aerosols may be particularly effective in direct animal to animal transmission (Wathes *et al.*, 1988).

Contaminated farm animal excrement will naturally enter the environment where it may survive for a considerable period and help perpetuate the disease. This has been noted in numerous epidemiological investigations where soil, water and slurry have been implicated (Gibson, 1965; Williams, 1975; Sojka *et al.*, 1977).

Wild animals have frequently been blamed for spreading a variety of diseases including *Salmonella*. However direct transmission is difficult to prove and rarely documented. It may be transmitted indirectly by faeces which, once deposited in the environment, may desiccate and allow salmonellae to survive for long periods in dust (Bate & James, 1958) or enter waterways, especially after a period of rainfall, and spread in this way (Haddock & Malilay, 1986).

Like the domestic variety, wild animals are capable of infecting others, particularly those in close proximity, as evidenced by the same organism being found in parents and their young (Schnurrenberger *et al.*, 1968; Singh *et al.*, 1980; Kirkpatrick & Colvin, 1986). Carnivorous animals and carrion scavengers tend to carry salmonellae more often than herbivores (Watts & Wall, 1952) and aquire it from their prey (Ostrolenk & Welch, 1942; Kirkpatrick & Trexler-Myren, 1986). *Salmonella* has been found in pigeon ticks, and has been shown to persist for several weeks in ticks and rat fleas (Kramer, 1963). This has also been demonstrated on a lizard, where *S.ohlstedt* was isolated from both a cloacal swab of the lizard and a tick (Iveson *et al.*, 1969).

Natural fauna have been implicated as causes of salmonellosis in domestic animals and humans. As with domestic animals, evidence for direct transmission is rare. However infection of both humans and domestic animals can be mediated through food or water contaminated by animals, birds and insects (Watts & Wall, 1952). In one experiment flies were shown to transmit

sufficient salmonellae to food and water to infect other flies and mice (Ostrolenk & Welch, 1942). The flies were also infected from infected mice, and fly eggs gave rise to infected maggots, pupae and adults after being fed on contaminated mash. Due to their habit of frequenting both filth and kitchens, cockroaches have been closely scrutinised and have found to be naturally infected (Anon, 1949; Singh *et al.*, 1980) and excrete *Salmonella* for up to 40 days (Mackerras & Pope, 1948). Ants have also been found with the same serovar as rats caught in the same area (Singh *et al.*, 1980). Infected lizards were said to have been the vectors of salmonellosis in livestock (McInnes, 1971) and snakes in poultry (Hinshaw & McNeil, (1944) cited by Chiodini & Sundberg, 1981).

Rodents have historically been regarded as major disseminators of diseases such as *Salmonella* but there have been few proven cases. Of rat and mice droppings collected in bakeries throughout the USA, 1.2% were positive and dried droppings were still infective after 5 months (Welch *et al.*, 1941). Infected rodents were thought to be the culprits in an incident on a poultry farm (Goyal & Singh, 1970).

Avian excretors propagated an outbreak in sheep by fouling water troughs (Watts & Wall, 1952). *Salmonella* was isolated from a number of carnivorous birds but absent in non-carnivorous varieties, indicating the role of the former in its epidemiology. Gulls are notorious in the pollution of drinking water reservoirs (Fennell *et al.*, 1974; Benton *et al.*, 1983) and have also caused outbreaks in cattle by polluting pasture (Johnston *et al.*, 1981; Reilly *et al.*, 1981).

Surveys of wild animals have identified reptiles as prominent carriers of *Salmonella* and *Arizona* strains (Iveson *et al.*, 1969; Kaura *et al.*, 1970; Koopman & Janssen, 1973; Iveson & Hart, 1983; Gugnani *et al.*, 1986). There is circumstantial evidence of people in New Zealand being infected with *S.saintpaul* by lizards (De Hamel & McInnes, 1971). Human infection rates were highest in areas where carriage of this serovar by skinks was highest. Good correlation between human and reptilian serovars has also been reported in Panama (Kuorany *et al.*, 1976) and Africa (Goyal & Singh, 1970). Others, (Hinshaw & McNeil, (1944), and Baker *et al.* (1972) cited by Chiodini & Sundberg, 1981) also report lizards and snakes as the source of human salmonellosis

The role of an environment contaminated with salmonellae is often overlooked in epidemiological investigations. Polluted water and contaminated soil have been significant in some circumstances.

Incidents of human salmonellosis have been described in Scotland (Reilly *et al.*, 1981) where drinking from, or bathing in, rivers polluted by sewage was implicated. Large water-borne outbreaks have been reported in Canada (Todd, 1989) and in California (Collaborative Report, 1971) involving reticulated supplies. Smaller incidents may go unreported or not be investigated and may contribute significantly to sporadic water-borne cases. For example, rainwater contaminated during collection on a roof caused a smaller outbreak (Ang *et al.*, 1973). Infections have also been postulated from contaminated aerosols (Darlow & Bale, 1959; Haddock & Malilay, 1986) and dust (Bate & James, 1958; Datta *et al.*, 1960).

Contamination of food from environmental sources has been noted. A close relationship between serovars isolated from vegetable crops and the water used to irrigate them was established in a Spanish survey (Garcia-Villanova Ruiz *et al.*, 1987) but their relationship to prevalent human cases was less clear. Such contamination may be a problem in salad crops which are not normally cooked: in an Italian survey approximately 70% of lettuces and fennel at retail outlets were contaminated with *Salmonella* (Ercolani, 1976).

Gulls which frequent sewerage works harbour a range of serovars very similar to that of the sewage on which they feed (Fenlon, 1981, 1983). The proportion of gulls excreting salmonellae was found to be higher closer to the sewage outfalls (Fenlon, 1981) but in contrast a survey of other species of birds from sewerage treatment works showed a very low incidence (Plant, 1978). A close relationship was found between serovars in wild birds in the vicinity of a polluted waterway in Hamburg and those in the water (Muller, 1965). Salmonellae found in free-flying birds in India were likely to have originated from human or domestic animal sources as judged by their patterns of antibiotic resistance (Sharma *et al.*, 1980).

Several outbreaks in stock have been due to environmental pollution (Reilly *et al.*, 1981). In some cases livestock drank directly from water polluted by sewage or abattoir effluent. In several others they had access to land contaminated by flooding or septic tank discharge, or on which sludge from sewage works had been spread. The spreading of sewage sludge on pasture may not always cause noticeable infection or outbreaks on grazing animals, as

was seen in one survey (Linklater *et al.*, 1985), although there was good correlation between the species of *Salmonella* isolated in sewage and from the local abattoirs.

Traditionally the transmission of *Salmonella* to farm animals has been regarded as faecal-oral and has generally been associated with contaminated feed. This led to a proliferation of animal feeds surveys, which frequently isolated the same *Salmonella* serovars in stock and feeds (Newell *et al.*, 1959; Galbraith, 1961; Taylor & McCoy, 1969). Among the most implicated feeds were meat, bone and fish meals, which can average 1-100 salmonellae/g (Taylor & McCoy, 1969). A survey of rendered feed meals of animal origin has recently been undertaken for all such plants in Tasmania (Holm, 1990). The *Salmonella* isolation rate of 2.6% compares most favourably with similar surveys of feather, meat and bone meal produced on mainland states between 1955 and 1979, which ranged from 40 to 91%.

ISOLATION METHODS

In isolating *Salmonella* from animals the type of specimen cultured can result in significant differences in the percentage of *Salmonella* carriers identified. The proportion of animals with *Salmonella* appears highest when mesenteric lymph nodes are cultured rather than faecal samples or other organs (Smith, 1959; Heuschmann-Brunner, 1974; Williams & Bellhouse, 1974). This has accounted for apparent differences in carriage rates of 2-fold in horses (Morse *et al.*, 1976), 5 to 9-fold in dogs (Smith, 1959; Khan, 1970), 4-fold in mink (Williams & Bellhouse, 1974), and 5-fold in cats (Smith, 1959).

The use of stool specimens rather than rectal swabs result in improved isolation rates (McCall *et al.*, 1966), although comparable results have been reported (Shaughnessy *et al.*, 1948). Two consecutive stool specimens were required to detect *Salmonella* in 100% and 87% of short and long term excretors respectively, compared with 3 and 9 consecutive rectal swabs. In pigs the isolation rate from caecal faeces was quadruple that of caecal swabs (Newell *et al.*, 1959). A similar survey of quokkas revealed swabs to be significantly less effective where *Salmonella* concentrations were below 10^3 per gram of faeces (Hart *et al.*, 1982).

Culturing other organs has met with mixed success. In fish the next most infected organ after the intestine is the liver, followed by the spleen and kidney

(Heuschmann-Brunner, 1974). Turtles appear to have only the faeces infected (Izadjoo *et al.*, 1987), whereas the spleen of guinea pigs (Moore, 1957) and the liver of frogs (Ang *et al.*, 1973) is more commonly and heavily infected than their respective intestines.

Salmonella isolation from carcasses is variable according to the processing and sampling methods. Certain areas of the carcass, such as the neck flap of chickens are more frequently contaminated with *Salmonella* due to the high level of water uptake by exposed connective tissue (Thomas & McMeekin, 1981). Swabs from the cervical area of the neck and the anal region gave more representative results for pig and cattle carcasses (Weissman & Carpenter, 1969).

Delayed testing of the specimens for up to three days appeared to have little significance on the result (McCall *et al.*, 1966). Some comparative differences in recovery from fresh and stored specimens were noted (Harvey & Price, 1983) indicating a possible reduction in the numbers of culturable salmonellae during storage. Improvements have been made to overcome this effect using two different strategies. Specimen transport media to maintain bacterial viability and resuscitation media to recover sub-lethally damaged bacteria have been used effectively (Harvey & Price, 1979).

The media used and conditions of incubation can also cause differences in the numbers and types of salmonellae isolated. It has been said of microbiologists that they would rather use another microbiologist's toothbrush than their methods! Use of the many and varied selective media, period and temperature of incubation and ancillary methods used in resuscitation or in the detection of multiple serovars make comparisons of surveys difficult. In general, the more methods used in parallel, the greater the *Salmonella* isolation rate and the range of serovars encountered (Harvey & Price, 1979). For economic reasons a combination of two selective enrichment broths and two selective agars is generally applied.

EFFECTS OF ENVIRONMENTAL CONDITIONS ON GROWTH AND SURVIVAL

The ability of bacteria to flourish in favourable conditions or survive longer in harsh ones is dependent on both the strain and the actual environmental conditions, such as temperature, pH, water activity, sunlight, nutrient

availability and the presence of pathogenic, predatory or competitive microorganisms.

Survival

Many workers have investigated the survival of *Salmonella* in many different circumstances. Some experiments were performed in carefully controlled situations where the effect of varying one parameter could be observed. Others used more natural environments where a complex interplay of factors was involved. The former type is useful to determine theoretical limits where salmonellae can be expected to be destroyed, and has been most useful to the food industry. The latter has generally been undertaken as part of epidemiological investigations where prolonged survival of the organism may explain recurrence of disease.

In some environments *Salmonella* has been shown to survive for great periods of time. In such circumstances its persistence may be an ongoing source of infection. Survival of up to four years has been reported on naturally contaminated chick fluff (Miura *et al* (1964) cited by Mitscherlich & Marth, 1984) and over two years in sewage (Rochaix (1930) cited by Mitscherlich & Marth, 1984). *Salmonella* was recovered after several months in slurry (Best *et al* (1971), Thunegard (1975) cited by Mitscherlich & Marth, 1984), sludge (Muller & Strauch (1968) cited by Mitscherlich & Marth, 1984), soil (Thunegard (1975) cited by Mitscherlich & Marth, 1984) and faeces (Drescher & Hopfengarter (1933) cited by Mitscherlich & Marth, 1984), compared with days to weeks on dried surfaces (Enkiri & Alford, 1971) and in water (McFeters *et al.*, 1974, Drescher & Hopfengarter (1933) cited by Mitscherlich & Marth, 1984).

Decreased survival of bacteria at progressively higher temperatures is a well known effect at temperatures above freezing point. *Salmonella* has been demonstrated to survive longer when chilled rather than at room temperature in pig and cow slurry (Thunegard (1975) cited by Mitscherlich & Marth, 1984), faeces (Kligler (1921) cited by Mitscherlich & Marth, 1984) and on vegetable surfaces (Felsenfeld & Young, 1945). Its survival at higher temperatures is much shorter. Six serovars were able to be recovered from sludge stored at 10-18°C for between 264 and 344 days, whereas none survived longer than 26 days at 30-35°C storage (Muller & Strauch (1968) cited by Mitscherlich & Marth, 1984).

Hydrogen ion concentration may affect the survival period of salmonellae. In an experiment (Muller (1965) cited by Mitscherlich & Marth, 1984) various types of animal faeces were inoculated with approximately 10^5 *Salmonella* cells and their survival at room temperature was observed. It was still recoverable after 60 days from the pig, cow, goat, horse, chicken, rabbit, turtle and rat faeces, all of which were alkaline. In all the acid faeces, from duck, swallow, carp and gull, survival was diminished to between 27 and 42 days. The persistence of each of four serovars was longer in pig slurry at a pH of 7.0 than in cow slurry of pH 6.7 under both chill and ambient temperature storage (Thunegard (1975) cited by Mitscherlich & Marth, 1984). In another investigation (Felsenfeld & Young, 1945) the survival of four salmonellae was monitored on vegetable surfaces held both on ice and at ambient temperature. All persisted longest on peas and beans which were the only alkaline vegetables tested. Similarly *S.typhi* was found to survive longer in a soil of pH 6.4-6.5 than when the pH was 4.8-5.0 (Kligler (1921) cited by Mitscherlich & Marth, 1984).

Environmental relative humidity (RH) can exert a pronounced effect on the viability of Gram negative bacteria on exposed surfaces (Mitscherlich & Marth, 1984). This has been shown for *S.derby* which survived over 7 days at 11% RH but less than 2 days at 53% RH (McDade & Hall (1964) cited by Mitscherlich & Marth, 1984). The water content of faeces may also be a factor in survival. *S.typhi* persisted between 1 and 3 days in liquid faeces in comparison with 3-8 days in pulpy faeces (Kligler (1921) cited by Mitscherlich & Marth, 1984). The converse was seen in soil and sand where the same serovar was able to be recovered after longer periods from moist rather than dry soil (Dempster (1894) cited by Mitscherlich & Marth, 1984).

The resident microbiota can also reduce the longevity of introduced bacteria. This effect has been demonstrated with enteric bacteria in estuarine water (McCambridge & McMeekin, 1981) and with *S.typhi* in sea water held at 10°C (Beard & Meadowcroft (1935) cited by Mitscherlich & Marth, 1984). It has also been shown that the active indigenous microbiota of compost establishes a homeostatic barrier to colonisation by *Salmonella* to the point where an inoculum of 100,000/g was eliminated after 6 weeks (Hussong *et al.*, 1985).

There appears to be considerable variability in the survival of different strains of *Salmonella* in various environmental conditions. In general *S.typhi* does not fare as well as other serovars, which appear quite variable in this trait according to the particular conditions. This has been shown for poultry faeces stored at

20°C, where an inoculum of 10^8 cells/g survived between 11-14 and 22-25 days depending on the serovar (Strauch & Muller (1968) cited by Mitscherlich & Marth, 1984). Similar findings have been observed in several other environments. In liquid manure stored at both 4 and 20°C survival varied between 10-14 and 18-21 days, and 35-42 and >63 days respectively (Kovacs & Tomasi (1979) cited by Mitscherlich & Marth, 1984). Some serovars persisted in sludge for between 264 and 344 days when held at 10-18°C while the range varied from <10 to 21-26 days at 30-35°C (Muller & Strauch (1968) cited by Mitscherlich & Marth, 1984). Cow slurry kept between 4.5 and 13.5°C was lethal to some strains after 92 days while others were still present after 286 days (Best *et al* (1971) cited by Mitscherlich & Marth, 1984). In pig slurry four serovars survived between 10-12 and 25-27 weeks chilled and between 12-14 and 20-22 weeks at 18-20°C (Thunegard (1975) cited by Mitscherlich & Marth, 1984). *S.typhi* was able to survive for only four days on autoclaved loam and sandy loam but three other serovars were still recoverable at 30 weeks (Papaconstantinou *et al* (1981), Papavassiliou & Leonardopoulos (1978) cited by Mitscherlich & Marth, 1984).

Growth

Temperature is a major factor controlling microbial growth. The temperature range of growth of 40 serovars was cited as between 6.5 and 47.0°C (Mitscherlich & Marth, 1984). The minimum growth temperature varied between 6.5 and 8.0°C. Only two of those tested, *S.braenderup* and *S.san-diego*, would not grow below 8.0°C. The range of maximum growth temperatures was 45-47°C, with four serovars unable to grow above 46°C; *S.gallinarum*, *S.pullorum*, *S.typhi* and *S.worthington*. Somewhat lower minimum growth temperatures have been reported elsewhere (Matches & Liston, 1968) where serotypic variation was between 5.5 and 6.9°C.

Rather less data is available on the pH limits for growth of salmonellae. The minimum growth pH was found to vary between 4.5 and 5.6 among the six serovars tested (Wethington & Fabian (1950) cited by Mitscherlich & Marth, 1984). The acid used to modify the pH of the growth medium has a bearing on the minimum pH for growth. When adjusted with hydrochloric or sulphuric acids the minimum growth occurred at pH 3.0-3.5 compared with 4.0-4.5 for acetic and lactic acids (Schwerin (1962) cited by Mitscherlich & Marth, 1984).

The effect of any limiting parameter on growth may be complicated by the interaction between other factors. For example, the salt concentration at which salmonellae can grow increases when the incubation temperature is increased towards that of optimum growth (Matches & Liston, 1972). Similarly, growth can be extended to lower pH environments when warmer conditions prevail (Ferreira & Lund, 1987).

RELATIONSHIP BETWEEN *SALMONELLA* AND INDICATOR BACTERIA IN WATER

The relationship between bacterial water quality and gastroenteritis has been investigated in several surveys and reviewed (Dufour, 1984). Of all the indicator bacteria tested, enterococci seem to correlate best with human gastroenteritis in recreational waters. This may be because enteric viruses are probably the main cause of water borne gastroenteritis and they can survive for long periods in the aquatic environment. Enterococci generally fare much better than Enterobacteriaceae in water and, as they are excreted at comparable levels, offer a better index of recreational water quality. Traditionally, faecal coliforms or *E.coli* have been used as indicators of faecal pollution where enteric bacterial pathogens, such as *Salmonella*, are investigated. In sediments the survival of salmonellae closely parallels that of faecal coliforms (Van Donsel & Geldreich, 1971), which provide a useful indicator. In Tasmania, potable water is assessed by coliform and faecal coliform levels, whereas faecal coliforms and faecal streptococci are used to monitor polluted waters using NH&MRC guidelines (NH&MRC & AWRC, 1987).

Considerable variation in the *Salmonella* : faecal coliform (FC) ratio has been reported; $1:10^1$-10^4 in surface waters and $1:10^3$-10^7 in sewage (McNeill, 1985). *Salmonella* was usually cultured from 100mL of polluted river water when >2000 FC/100mL were present (Menon, 1985). However, two isolations were noted in water containing approximately 230 FC/100mL. More extensive investigation has verified this finding (Geldreich (1976) cited by Menon, 1985), in which salmonellae were isolated in under 28% of waters containing less then 200 FC/100mL. The proportion rose to 85% and 98% where faecal coliform concentrations were 200-2000 and >2000 per 100mL respectively. This relationship may vary according to other variables. The source of pollution may be significant In one survey, where wild animals were the major contributors to

water-borne pollution, salmonellae were frequently encountered in waters containing <200 FC/100mL (Wright, 1982). This effect was also documented in West Australia where *Salmonella* were frequently observed in natural waters devoid of indicator bacteria (Iveson & Fleay, 1991). Analysis of sediments has also been used as a guide to water quality. The concentration of *Salmonella* in sediments exceeded that of the overlying water by a factor of 10^2-10^3 in fresh water (Van Donsel & Geldreich, 1971) but this relationship is not valid in marine situations (McNeill, 1985).

Pathogen : indicator ratios in sewage were influenced by differential survival during treatment, incidence of excretors in the population and pollution from point sources as well as test methodology and sample volumes (McNeill, 1985). In disinfected sewage the likelihood of *Salmonella* isolation was increased 100-fold that of untreated sewage of comparable faecal coliform status (Kott (1977) cited by McNeill, 1985). Salmonellosis outbreaks increased both the numbers of excretors and the level of *Salmonella* in faeces, both of which increased the *Salmonella* : FC ratio of raw sewage. Examination of effluent from a chicken processing factory demonstrated a consistent relationship between faecal coliforms and salmonellae of >1 *Salmonella* /500 FC (Hoadley *et al.*, 1974). It was valid over the range of faecal coliforms seen in raw to secondary treated effluent. Corresponding faecal streptococci levels showed poor correlation.

Differences in test methodology and sample volumes used make comparisons between some surveys difficult. Until recently, the most probable number (MPN) technique has been the only method capable of enumerating *Salmonella* in specimens contaminated with other enteric bacteria. Such methodology is cumbersome and most workers have resorted to a test giving a qualitative result from a given sample. The problem is one of sample variability; different volumes of water, sediment samples and Moore swabs have all been used, making comparisons between surveys difficult. Recent techniques (Desmonts *et al.*, 1990; Knight *et al.*, 1990) of direct microscopic counting are capable of enumerating salmonellae in water and wastewater. However, they can detect cells which are not culturable by traditional methodology. This poses a further problem; what is the significance of these forms? Viable non-culturable (VNC) forms of *E.coli* and *Salmonella* have been reported after exposure to various forms of stress including nutrient starvation (Xu *et al.*, 1982), chlorination (Desmonts *et al.*, 1990), and sunlight (Davies & Evison, 1991) and was postulated as a survival mechanism by Xu *et al*, (1982). They have been reported to revert if placed in conditions favourable to their recovery, such as

unchlorinated water (Desmonts *et al.*, 1990) or the mammalian gut (Singh *et al* (1986) cited by Desmonts *et al.*, 1990). The significance of VNC *Salmonella* may differ under certain conditions. Enteric bacteria, including *Salmonella* persist longer in fresh water than in sea water when quantified by traditional culture methods (Davies & Evison, 1991). No such difference was apparent when VNC forms were enumerated, suggesting *Salmonella* transformed to the VNC form more rapidly in sea water.

SUMMARY

The incidence and epidemiology of salmonellosis in Tasmania was investigated initially. The incidence of human salmonellosis in Tasmania was compared with that of other Australian states. There appears to be no significant differences between the incidence of salmonellae in Tasmania and Australia except in the distribution of *Salmonella mississippi* which is very abundant in Tasmania but rare elsewhere.

The disparity between the incidence of *S.mississippi* in Tasmania and mainland Australia may be caused by a number of factors. *Salmonella mississippi* may be:-

- transmitted by a contaminated food produced in Tasmania and consumed locally.
- a predominantly water-borne infection.
- caused or exacerbated by some aspect of the Tasmanian population.

Seasonal, geographic and demographic distributions were determined to highlight any potential problem areas or differences between *S.mississippi* and other *Salmonella* spp. The disease pathology was investigated to determine whether some aspect of the human population is similarly implicated.

As the occurrence of *Salmonella mississippi* is an ongoing phenomenon in Tasmania it seems reasonable that an animal reservoir is involved. As a consequence indigenous animals were investigated in an attempt to identify a specific animal reservoir or ecological niche which may help perpetuate *S.mississippi* in Tasmania. This required both recognition of salmonellosis and

an understanding of the mechanisms by which salmonellae are transmitted from infected individuals to human and animal populations.

Another aspect which may be of significance to the abundance of *S.mississippi* in Tasmania is its behaviour in the environment. If *S.mississippi* was better suited than other salmonellae to growth or survival in local environmental conditions this may enhance its persistence in Tasmania.

MATERIALS AND METHODS

SALMONELLA SURVEYS

Surveys were undertaken for several distinct purposes. The rate of human salmonellosis in Tasmania was determined from a survey of the local asymptomatic population. General surveys of food, animals and water were carried out in an attempt to locate a common source of *Salmonella mississippi*. An area in which the native animal population was found to be infested with *S.mississippi* was intensively investigated to understand its ecology better. Water was surveyed to assess the effectiveness of indicator bacteria to predict contamination with *Salmonella*.

Several different specimen types were tested in the *Salmonella* surveys and included:

- Foods.
- Waters.
- Animal faeces.
- Animal intestinal segments.
- Cloacal / rectal swabs.
- Human faeces.

ANALYSIS OF *SALMONELLA* SURVEY DATA

Comparison of human salmonellosis case rates between Australian states and age and sex variations of salmonellosis were made using notifications on the National *Salmonella* Surveillance Scheme (NSSS) data base. Although some entries from this source were duplicated due to clerical errors, this data should suffice for interstate comparisons provided that errors were randomly distributed among the states.

Tasmanian NSSS records were carefully examined as part of this investigation and were modified to remove duplications by cross-checking entries with patient records or Health Department notifications. The corrected data was used whenever exclusively Tasmanian salmonellosis was being considered and no comparisons were made with Australian statistics.

Monthly occurrence of *S.mississippi* and other *Salmonella* spp. in Tasmania was calculated from corrected NSSS data over the 1980-1990 period.

Human faecal specimens were tested for one of three reasons. Samples received in the laboratory for screening of groups of asymptomatic people for unrelated reasons were also tested for the presence of *Salmonella* to determine the abundance of *Salmonella* in the asymptomatic population. Approximately half of this sample provided three specimens at weekly intervals. The incidence of *Salmonella* in diarrhoea was determined by testing faeces from diarrhoeal patients of unknown aetiology and no links with known *Salmonella* patients. Finally, known *Salmonella* cases were investigated to determine the likely source of infection and its spread to familial and other contacts.

Age and sex distributions of human salmonellosis in Tasmania were calculated from corrected NSSS data and the Australian Bureau of Statistics population data. The age ranges used were 0, 1-2, 3-4, 5-9 and subsequent ten-year groups. The midpoint age ranges were plotted using Harvard Graphics software package.

Regional variation in human salmonellosis was examined for 18 regions in Tasmania. Population statistics were obtained from the Australian Bureau of Statistics 1987 preliminary figures (Jackson, 1988). The source of the *Salmonella* data was the NSSS data base which was subsequently corrected for duplications and familial outbreaks. Several records did not give the patient's address and were distributed proportionally to the regions serviced by the notifying laboratory. The mean incidence of human salmonellosis and its standard deviation were calculated for each region from corrected NSSS data over the 11 year period (1980-1990).

Statistical analysis on the above was carried out on differences between means using the one tailed test (Clarke, 1969) using P values derived from chi-squared tables.

The distribution of *Salmonella* serovars among various specimen types in Tasmania was assessed using empirical data from all available sources including: Department of Primary Industry (DPI) Mt. Pleasant Laboratory, Institute of Medical and Veterinary Science (IMVS) annual reports (1980-89), National *Salmonella* Surveillance Scheme (NSSS) information (1980-90) as well as this investigation. Correlation of serovars isolated from various sample types was made using Krylov's coefficient or the coefficient of Fager & McGowan (Legendre & Legendre, 1983)

SAMPLING

Food samples were taken either as part of a survey of locally produced foods or as a result of epidemiological investigations of salmonellosis. Sampling was of either the whole food item or a sample unit of not less than 100g.

Water samples were taken in sterile 250mL sample bottles containing 0.25mL of 10% sodium thiosulphate solution to neutralise chlorine in the sample. Bottles used to collect sewage samples also contained 0.25mL of 10% EDTA solution to chelate any heavy metals present, and so neutralise their possible toxicity on bacteria.

Moore swabs were also used to examine water for *Salmonella* and were prepared using cotton gauze pads which were folded and stapled with steel staples and sterilised by autoclaving at 121°C for 15 minutes. Swabs were attached to nylon fishing line and placed in stream flow for two to seven days before recovery. On collection the swabs were cut from the line and placed into sterile plastic bottles containing about 30mL of stream water to keep the swab moist.

Animal faecal specimens were obtained by one of three methods as appropriate;

- collection of fresh faecal pellets from the natural habitat.
- trapping of live animals in possum (cage) traps provided by the David Collins Leukaemia Foundation where scats or rectal / cloacal swabs could be taken prior to release.
- collection of intestinal segments from recent road kills and fish obtained by the electrode stun method.

Human faecal specimens were obtained in faecal pots from cooperative *Salmonella* patients, their families and contacts and from asymptomatic people who were being screened for unrelated purposes.

Permission to obtain animal specimens was obtained from the Department of Lands, Parks, Wildlife and Heritage. Approval was obtained from the Animal Ethics committee of the University of Tasmania for all research involving native animals. Permission to investigate human salmonellosis patients was obtained from the Director of Public Health and the medical practitioner handling each patient. Fish specimens were taken by and with the approval of the Department of Inland Fisheries.

All specimens were transported to the laboratory in insulated containers at temperatures below ambient and, wherever possible, at 4°C.

SALMONELLA ISOLATION METHODOLOGY

The methodology used in the isolation of *Salmonella* was dependent on the type of specimen being tested. Different isolation protocols were employed for

- foods.
- waters.
- Moore swabs, soil and non-filterable water.
- animal faeces, intestinal segments and cloacal swabs.
- human faeces.

respectively.

All microbiological media formulations and commercial details are given in Appendix 1.

RESUSCITATION

A resuscitation step was included as foods, waters, soil and specimens from animals were presumed to have been subject to conditions which could cause sublethal injury to any *Salmonella* present. Resuscitation was effected by mixing the specimen with sterile buffered peptone water. The period of incubation was dependent on the specimen type.

Foods other than lettuces and poultry were homogenised in a blender (Sorvall, Du Pont Instruments) and 25g added to 225mL of buffered peptone water. Poultry carcasses were rinsed with 225mL of 0.1% peptone water and the fluid drained off served as the sample. Lettuces were washed in buffered peptone water which was retrieved by draining. Incubation of the resuscitation broth was 18 - 24 hours at 37°C.

Moore swabs, soil and water too turbid to pass through a 0.45 *u*m membrane filter were added to 225mL of buffered peptone water and incubated at 37°C for 18 - 24 hours.

Water was filtered through a 0.45 um membrane filter and the membrane added to 20mL of buffered peptone water and incubated at 37°C for 18 - 24 hours.

Animal faeces were made into a paste with a small amount of sterile distilled water to homogenise the sample. Approximately one gram was added to 20mL of buffered peptone water and resuscitated at 37°C for 4 hours. The homogenised paste was also used to streak selective media (direct plating).

For freshly killed animals, a section of both the small and large intestine was removed, cut into approximately 1cm sections and blended with about 50mL of buffered peptone water in a blender (Sorvall, Du Pont Instruments). This was used to inoculate the selective media used for direct plating and the remainder was added to 225mL of buffered peptone water and incubated at 37°C for 4 hours.

Cloacal or rectal swabs were added to 20mL of buffered peptone water, mixed by vortex and incubated at 37°C for 4 hours.

Human faecal specimens were processed within 8 hours, eliminating the need for a resuscitation step. Faeces were made into a paste with a small amount of sterile distilled water to homogenise. This was used to streak the selective media directly and to inoculate the selective enrichment broths.

SELECTIVE ENRICHMENT

Several different selective enrichment broths were used in the selective enrichment step, depending on the type of sample. Rappaports RV Broth (RVB) was used in conjunction with either Mannitol Selenite Cysteine Broth (MSCB) or Selenite Broth (SB). The isolation protocol for food samples used MSCB and RVB; that for all other sample types used SB and RVB.

In the case of freshly obtained faecal specimens the faecal paste was inoculated directly into the selective broths. Otherwise 1mL of the resuscitation broth was added to SB and 0.1mL added to RVB. Incubation was for 18 - 24 hours at 42°C for MSCB and SB and at 37°C for RVB.

SELECTIVE PLATING

At least two different types of selective media were inoculated from each selective enrichment broth and for direct plating of every sample. Desoxycholate Citrate Agar (DCA) and Xylose Lysine Desoxycholate Agar (XLD) were used for every sample. In addition Hektoen Enteric Agar (HEA) was also used on some samples and MLCB Agar was used for dairy food tests to detect

lactose-fermenting salmonellae. Each selective enrichment broth was streaked onto selective media which were incubated at 37°C for 18 - 20 hours.

PURIFICATION, SCREENING TESTS AND CULTURE IDENTIFICATION

Two of each suspect *Salmonella* morphotype from each selective medium were purified by streaking onto Cystine Lactose Electrolyte Deficient Agar (CLED) and incubating for 15-18 hours at 37°C. Lactose fermenting colonies (yellow), apart from those on MLCB Agar cultured from dairy foods, were discarded. An oxidase test was used as a screening test at this stage; oxidase positive isolates were discarded.

As *Proteus* spp. were frequently isolated from many specimens and appeared similar in morphology to *Salmonella* spp. the urease reaction was also included in the screening tests. The culture was inoculated onto a Christiansen urea agar slope and incubated at 37°C. A pink colouration appearing within 4 hours is a presumptive positive reaction for *Proteus* sp.; such culture were discarded. Some salmonellae give a positive urease reaction but only after a longer incubation period.

Urease and oxidase negative cultures were further screened using API-Z rapid screen kits (API, Montalieu Vercieu, France). The "A" well of the API-Z was rehydrated with a drop of sterile distilled water. This was inoculated with a colony removed from the CLED plate with a sterile applicator stick and was incubated for 2 - 3 hours at 37°C. The evolution of a blue colour or no colour change indicated a suspect *Arizona* sp. or *Salmonella* sp. respectively. If, after the addition of a drop of Fast Violet B reagent, the well changed from white to red, the probability of the isolate being *Salmonella* sp. was increased.

Suspect *Salmonella* isolates were identified using the API-20E test kits (API, Montalieu Vercieu, France). A colony from the CLED plate was suspended in 5mL of sterile distilled water and used to inoculate the API-20E test strip. If, after incubation at 37°C for 18 hours, the isolate was identified as *Salmonella* sp. or *Arizona* sp. it was streaked onto a Nutrient Agar (NA) slope and a Blood Agar (BA) plate. After incubation overnight at 37°C the BA culture was used to determine the serology using the range of commercially available *Salmonella* agglutination antisera (Wellcome Australia).

SEROLOGICAL EXAMINATION

A small part of a one day old colony was removed from a non-selective agar medium and mixed with a drop of physiological (0.85%) saline on a clean microscope slide until an even suspension was obtained. This was examined for auto-agglutination before proceeding. A loop of *Salmonella* agglutinating antiserum was then mixed for about 20 seconds with the suspension on the slide and examined for an agglutination reaction. Of the commercially available range of *Salmonella* antisera, polyvalent O was tested first and, if agglutination was observed, the remaining O antisera were tested. If the culture agglutinated with 13,22-O antisera then it was tested against the flagellar antisera b-H and 1,5-H, the flagellar antigens of *Salmonella mississippi*. If one of these antigens was present an attempt to change the phase of the H antigen was made using the ditch plate method (Collins & Lyne, 1970).

For confirmation of *Salmonella* serovars the NA slope culture was sent to the Salmonella Reference Laboratory at the Institute of Medical and Veterinary Science (IMVS) in Adelaide, Australia. Some serovars, which can be further subgrouped by phage type, were sent the Melbourne Diagnostic Unit (MDU) of the Microbiology Department at the University of Melbourne for phage typing. Accompanying the culture were details of isolation to be included in the National *Salmonella* Surveillance Scheme (NSSS).

EXAMINATION FOR MULTIPLE *SALMONELLA* SEROVARS

Examination for multiple *Salmonella* serovars was carried out immediately when *S. mississippi* was isolated. However with other serovars the process was delayed until the serological confirmation was available from the reference laboratories.

In cases where *S. mississippi* was the only isolated serovar, examination for other serovars could be carried out. A suspension was made by taking a swab from the selective agar and suspending in Ringer solution. The suspension was used to inoculate a filter paper bridge containing both b-H and 1,5-H antisera on a ditch plate on the same type of agar the suspension was made from. The same procedure was used as for the phase change operation. Typical colonies were purified on CLED and screened and identified as previously described.

In cases where other serovars were isolated, the selective agar culture was suspended in nutrient broth containing 15% glycerol and frozen until the

serology was reported. Then, if all the H-antisera were available, a similar process was followed using the thawed suspension and a mixture of the appropriate H-antisera. In cases where flagellar antisera to any of the H-antigens of the isolate was not available examination for multiple serovars was not practical.

ANTIBIOTIC SENSITIVITY TESTING

Antibiotic sensitivity patterns are useful in epidemiological investigations to separate unrelated cases and to help identify common sources. Cultures under investigation were grown on blood agar for 18-24 hours at 37°C. A small amount of culture was removed from isolated colonies and suspended in 2mL of 0.85% sterile saline. The suspension was spread onto Diagnostic Sensitivity Test (DST) agar using a sterile cotton swab, and up to six antibiotic discs were placed on the lawn using an Oxoid disc dispenser. The plates were incubated for 18 hours at 37°C. The radius of the zone of inhibition was used as a measure of the comparative antibiotic sensitivities of the cultures.

Antibiotic impregnated discs (Oxoid) of the following types were used:

N	Neomycin	30 ug
P	Penicillin G	2 IU
TE	Tetracycline	10 ug
C	Chloramphenicol	30 ug
RL	Sulphamethoxazole	25 ug
K	Kanamycin	30 ug
S	Streptomycin	10 ug
CIP	Ciprafloxacin	5 ug
CL	Cephalexin	30 ug
AML	Amoxycillin	10 ug

ENUMERATION OF INDICATOR ORGANISMS IN WATER

Enumeration of coliforms, faecal coliforms (FC), and faecal streptococci (FS) was by the following membrane filtration technique.

Sample volumes of >20mL were measured directly into a filter funnel (Gelman Sciences) and then passed through a sterile, gridded, 47mm diameter cellulose nitrate filter (Gelman Sciences) of 0.45um pore size under vacuum. Sample volumes of <20mL were mixed with approximately 20mL of buffered dilution water before filtration. The filter was then placed on a 50mm petri plate of selective agar and incubated under the following conditions:

Coliforms were cultured on Membrane Lauryl Sulphate agar (mLSA) for 4 hours at 30°C followed by 16 hours at 35°C. Faecal coliforms were grown on mLSA for 4hr/30°C followed by 16hr/44.5°C. Both grow as yellow (lactose fermenting) colonies on this medium. Faecal streptococci were enumerated after culture on Membrane Enterococcus agar (mEA) at 37°C for 48 hours, on which they form dark pink - sepia colonies under such conditions. Plates containing between 10 and 100 colonies of the target organisms were counted as the number giving the best estimate of the actual levels in the water sample.

ANALYSIS OF RESULTS

The proportion of samples containing *Salmonella* was calculated for various ranges of indicator bacteria concentration. Ranges used were half log cycles of indicator organism concentration (0-0.49, 0.5-0.99, 1.0-1.49,...). The midpoint of the ranges were used to plot the data in the form of line graphs using Harvard Graphics software package.

ENUMERATION OF *SALMONELLA* AND FAECAL COLIFORMS IN FAECES

Fresh skink faeces was collected and immediately weighed into a sterile tared container using an analytical balance (Sartorius 2001 MP2). It was homogenised by vortex in sterile distilled water to give an initial concentration of 1:100 from which an aliquot was used to determine the pH. Subsequent serial decimal dilutions were prepared with 9.0mL Ringer solution. Enumeration of faecal coliforms was carried out as described for water (above). Salmonella was enumerated by similar membrane filtration technique but the membrane was incubated on XLD agar for 24 hours at 37°C after which time black centred colonies were counted and a representative number identified by serology.

TEMPERATURE CHARACTERISTICS OF *SALMONELLA MISSISSIPPI*

Salmonella mississippi was grown over a range of temperatures to determine the limits of its growth.

A temperature gradient incubator (Toyo Kagaku Sangyo Co. Ltd., Tokyo) was loaded with L-tubes containing 18mL of nutrient broth and was left overnight

for the temperatures to equilibrate. The tubes were inoculated with 0.2 mL of a culture of *Salmonella* sp. in logarithmic phase after being incubated in nutrient broth for 15 hours at 37°C. Growth was quantified by measurement of turbidity using a nephelometer (EEL, Unigalvo). On reaching 100% absorbance, 2mL of concentrated biogard was added to the L-tubes to kill the bacteria and to prevent adhesion to the inside of the tubes which enabled effective cleaning.

Growth curves were determined at various temperatures by plotting nephelometer absorbance against incubation time. The growth temperature characteristic (Fig.5) was determined by plotting the square root of the time (minutes) taken for the cultures to reach 50% absorbance against incubation temperature. The theoretical minimum growth temperature (T_{min}) was calculated by extrapolation using the method of Ratkowsky *et al* (Ratkowsky *et al.*, 1982) utilising the linear portion of the growth temperature curve. The minimum, optimum and maximum growth temperatures were also calculated from the whole curve using the method of Ratkowsky *et al* (Ratkowsky *et al.*, 1983).

Minimum growth temperatures were determined more accurately by incubation of pure cultures in nutrient broth maintained at low temperature in a Lauda RMS refrigerated water bath (Messgeräte-Werke Lauda, Lauda-Königshofen, FRG) which maintained temperature within 0.1°C. Approximately 10^4 cells/mL of *Salmonella* in logarithmic growth phase was incubated for up to three weeks at 7.0°C, 6.5°C, 6.0°C and 5.5°C. Growth was determined by comparison of the initial and final bacterial concentrations after the cells were resuspended by vortex mixing for 20 seconds. Enumeration was by the membrane filtration method, using 0.45*u* gridded membranes (Gelman Sciences) which were incubated at 30°C on nutrient agar plates and examined after 48 and 72 hours. The culture was considered to have grown if the final count exceeded the initial count by a factor of ten or above.

SURVIVAL OF *SALMONELLA MISSISSIPPI*

The ability of *Salmonella mississippi* to survive under various adverse conditions was compared with that of other salmonellae. The conditions included sterile distilled water and sterile sea water at various temperatures and sunlight conditions, and in soil.

INOCULUM PREPARATION

Salmonella cultures were maintained in cryogenic storage at -70°C in brain heart infusion broth. Log phase cultures were prepared by inoculation into nutrient broth and overnight incubation at 30°C. The suspensions were centrifuged at 1,000G for 15 minutes, the supernatant discarded and the cells resuspended in sterile water. The washing process was repeated three times. The inoculum was enumerated at the initiation of each survival experiment.

ENUMERATION

Serial decimal dilutions were prepared using 9.0mL of quarter strength Ringer solution and 1.0mL of the test suspension and vortex mixing for 10 seconds. Enumeration was by the membrane filtration technique, using buffered dilution water as a rinse fluid, through 47mm diameter cellulose nitrate membranes of 0.45uM pore size (Gelman Sciences). The membranes were placed on Nutrient Agar (NA) and incubated at 30°C for 3 days.

SURVIVAL IN STERILE FRESH WATER AND SEA WATER

A series of dilutions was made using quarter strength Ringer solution. Sterile glass bottles containing 20mL of water and sea water were inoculated with 1mL of the appropriate dilution to give approximately 10,000 salmonellae per mL. The bottles were stored in the dark at 4°C, 15°C and 30°C. The bottle contents were resuspended by vortex for 20 seconds before sampling.

SURVIVAL IN SUNLIGHT

Salmonella cultures were suspended in sterile water to simulate conditions in natural fresh water to investigate the effect of sunlight on the survival of *Salmonella* in drinking water. Washed cell suspensions of approximately 10^7 cells/mL sterile water were placed in sterile glass bottles in duplicate. One bottle was wrapped in foil to serve as a control, the other exposed to sunlight. The bottles remained outside in full sunlight for an entire day, after which their respective contents were enumerated by membrane filtration. The latitude in Hobart is 43°S and the experiments were conducted during June on predominantly overcast days where temperatures fluctuated between 4°C and 10°C.

ANALYSIS OF RESULTS

Percentage survival was used to measure lethal effects of sunlight exposure on various *Salmonella* strains and was calculated by:

$$\frac{\text{number of survivors in sunlight}}{\text{number in unexposed control}} \times 100$$

Natural die-off in the dark, due to temperature effects, was determined by comparison of bacterial numbers in the unexposed control with the original inoculum in order to establish the rôle of sunlight rather than temperature as the lethal agent.

Survival experiments conducted on the same day allowed direct comparisons between different strains. However, no direct comparisons were possible between trials conducted on different days because of variable levels of incident solar radiation.

SURVIVAL IN SOIL

Washed cell suspensions of several *Salmonella* serovars were prepared and approximately 10^8 cells were sprayed onto 100cm^2 plots of soil which were not shaded from direct sunlight. Duplicate cores of approximately 1cm^3 were removed at weekly intervals, mixed in 90mL buffered peptone water and tested for the presence of salmonellae using the enrichment method indicated previously.

PERSISTENCE OF *SALMONELLA* IN ANIMALS

To investigate the possibility of an affinity between certain animals and *Salmonella mississippi* several animal experiments were carried out.

In all three excretion trials the animals were housed in individual cages which were cleaned out daily to reduce the possibility of re-infection from contaminated faeces. They were fed on *Salmonella*-free food. Scats were collected at approximately weekly intervals and were immediately transported to the laboratory for culture.

In the first experiment, the duration of excretion of *Salmonella mississippi* was determined for five naturally infected native cats which were trapped at Judbury.

The other two excretion trials involved inoculation of eight native cats and six brush possums. The animals were tested to ensure they were not excreting *Salmonella* by culturing at least three fresh scats produced on separate days. Animals found to be excreting *Salmonella* were released. An inoculum was prepared by adding *Salmonella* grown in nutrient broth for 18 hours at 37°C to sterile milk to give a final concentration of approximately 10^7 *Salmonella* / mL. Animals were orally inoculated using a syringe containing 1mL of the milk suspension.

RESULTS

The results of surveys and growth and survival experiments are mostly set out in table form. They are more fully discussed in the discussion section.

INCIDENCE OF *SALMONELLA* IN THE HUMAN POPULATION

TABLE 4. SURVEY OF HUMAN FAECAL *SALMONELLA* IN TASMANIA

INVESTIGATION	Samples Tested	*Salmonella* Isolated	Isolation Rate
Asymptomatic people	758	1	0.13%
Diarrhoea of unknown aetiology	149	3	2.0%
Contact with *S.mississippi* case	62	13	21%
Contact with other salmonellae	65	19	29%
TOTAL	1034	36	

In a survey of the general population of Hobart 758 non-diarrhoeal stools were examined resulting in the isolation of one *Salmonella* (*S.mississippi*) and one *Shigella* (*Sh.flexneri* group 3b).

Of 149 patients with diarrhoea of unknown aetiology, three faecal specimens contained *Salmonella* (two *S.mississippi* and one *S.typhimurium*) and a further two contained *Campylobacter jejuni*.

Faecal testing of contacts of known *Salmonella* patients revealed 25% of the contacts tested to be excreting *Salmonella* also. One of this group was an asymptomatic excretor of *Shigella boydii*. It should be noted that several of the contacts were not familial and have been omitted from calculations of infection of close contacts in the discussion.

MONTHLY VARIATION OF HUMAN SALMONELLOSIS IN TASMANIA

The data presented in Tables 5 and 6 below originated from the NSSS data base which was subsequently corrected to remove duplications.

TABLE 5. MONTHLY ISOLATIONS OF HUMAN *SALMONELLA MISSISSIPPI* IN TASMANIA

year MONTH	80	81	82	83	84	85	86	87	88	89	90	Total	Monthly Proportion
Jan	3	4	3	3	4	3	1	1	18	3	8	51	15.6%
Feb	1	3	5	3	3	3	2	6	15	9	4	54	16.5%
Mar	3	2	7	9	1	6	5	8	9	23	12	85	26.0%
Apr			2	8	2	5	1	2	9	3	6	38	11.6%
May			1	2	2			1	2	4	5	17	5.2%
Jun			1	1			1	1	1	1	3	9	2.8%
Jul			1	2	1					1	3	8	2.4%
Aug	1			1					3			5	1.5%
Sep				1					1		1	3	0.9%
Oct		1			1							2	0.6%
Nov		1	2	2	2	1		4	1	2	5	20	6.1%
Dec	1		3	3	3	5	1	6	5	3	5	35	10.7%
TOTAL	9	11	25	35	19	23	11	29	64	49	52	327	

TABLE 6. MONTHLY ISOLATIONS OF OTHER HUMAN *SALMONELLA* SPP. IN TASMANIA

year MONTH	80	81	82	83	84	85	86	87	88	89	90	Total	Monthly Proportion
Jan	13	3	4	6	21	8	7	13	7	12	13	107	13.9%
Feb	5	4	4	29	8	5	9	20	3	10	8	105	13.7%
Mar	6	5	1	13	4	3	6	18	3	22	6	87	11.3%
Apr	4		5	4	5	1	3	8	3	12	7	52	6.8%
May		3	3	6	5	3	1	9	9	9	8	56	7.3%
Jun	2	1	4	1	1	12	3	12	1	10	6	53	6.9%
Jul	4	2	4	6	1	6	4	1	6	4	4	42	5.5%
Aug	6	2	1	2	2	4	1	3	4	3	6	34	4.4%
Sep	8	4	4	5	6	4	5	7	2	3	3	51	6.6%
Oct	2	3		12	1	4	3	4	1	9	3	42	5.5%
Nov	1	2	3	12	8	4	2	6	2	10	8	58	7.5%
Dec	1	5	5	13	6	4	3	4	10	17	14	82	10.7%
TOTAL	52	34	38	109	68	58	47	105	51	121	86	769	

REGIONAL VARIATION IN HUMAN SALMONELLOSIS IN TASMANIA

TABLE 7. REGIONAL HUMAN SALMONELLOSIS IN TASMANIA -

Map Ref	REGION	Popul^{n} (x1000)	*Salmonella* cases (1980-90) S.m	Total Sal.	Mean annual case rates / 10^5 S.m	Total Sal.	% S.m
A	Hobart	136.4	62	258	4.1	17.2	24
B	Southeast	8.7	9	40	9.4	41.2	23
C	S. midlands	17.5	2	24	1.0	12.4	8
D	Channel	25.1	14	49	5.1	17.8	29
E	Huon	8.6	3	11	3.3	11.7	27
F	Derwent	13.3	5	16	3.4	10.7	31
G	E. coast	8.3	9	16	9.9	17.6	56
H	N. midlands	21.9	18	115	7.5	47.7	16
I	Launceston	66.3	31	147	4.3	20.2	21
J	Northeast	21.9	20	47	8.3	19.5	43
K	Flinders Is.	1.0	2	10	17.5	87.4	20
L	King Is.	1.8	0	12	-	60.3	<8
M	W. Tamar	6.6	2	16	2.8	22.1	13
N	Mersey	39.0	86	150	20.0	35.0	57
P	Burnie	53.6	14	63	2.4	10.7	22
Q	Northwest	8.0	4	10	4.5	11.3	40
R	W. coast	10.9	3	15	2.5	12.5	20
S	Plateau	N/A	No permanent residents				
	Tasmanian total	449	284	852			
	Mean for Tasmania				5.8	17.3	33.3

S.m denotes *Salmonella mississippi*

Due to the low numbers of both *Salmonella* and humans in Tasmania the values of standard deviation and mean were comparable for all regions listed above. However, the high apparent incidence of *S.mississippi* salmonellosis in the Mersey region corresponds to an outbreak in the area during 1988. The apparently elevated incidence of human salmonellosis on the Bass Straight islands is probably an anomaly due to the very small populations of King and Flinders Islands. Another interesting fact is that all 12 salmonellosis cases on King Island were caused by *S.typhimurium*.

FOOD SURVEY

The results of examination of 653 foods are shown in Table 8. Only three food types yielded *Salmonella*. The highest incidence of *Salmonella* was recorded in chickens and other poultry with two positive samples from 67 oysters tested. The *Salmonella* serovars encountered in chicken have changed over the period of testing. Since it was first isolated in 1989 *S.sofia* has become the predominant serovar in Tasmanian produced chicken. *Salmonella hadar* first appeared in chickens during the fourth quarter of 1990 and was still persistent in mid 1991.

TABLE 8. *SALMONELLA* ISOLATED FROM TASMANIAN FOODS

FOOD TYPE	N° Tested	N° (+)	% (+)	SEROVAR	No.
Mushrooms	50	0	0		
Muttonbirds	16	0	0		
Chicken	42	*16	38	*S.muenchen*	(1)
				S.sofia	(8)
				S.hadar	(3)
				S.typhimurium-12a	(3)
				S.typhimurium-170	(1)
Other poultry	10	1	10	*S.typhimurium*-135	(1)
Oysters	70	2	3	*S.rowbarton*	(1)
				S.(IV) 43:d:-	(1)
				S.(rough):43:-	(1)
Other shellfish	21	0	0		
Lettuces	36	0	0		
Raw milk	43	0	0		
Eggs	23	0	0		
Sprouts	140	0	0		
Smallgoods	75	0	0		
Raw beef	39	0	0		
Wallaby meat	12	0	0		
Paté	76	0	0		
TOTAL	653	19	20 isolates of 9 serovars		

* One chicken contained both *Salmonella hadar* and *S.sofia.*

WILD ANIMAL SURVEY

Salmonellae were primarily isolated from faecal material of carnivorous and insectivorous indigenous animals. Two species, native cats and metallic skinks, frequently excreted *Salmonella mississippi* but rarely other serovars. Few herbivores carried *Salmonella*.

TABLE 9. *SALMONELLA* ISOLATED FROM TASMANIAN WILD ANIMALS

SPECIES	ANIMALS TESTED	NUMBER POSITIVE	SEROVAR	NUMBER ISOLATED
MAMMALS				
Barred bandicoot	1	1	*S.mississippi*	1
Bettong	3	0		
Brown bandicoot	4	*1	*S.mississippi*	1
			S.warragul	1
Brush possum	40	1	*S.mississippi*	1
Echidna	3	0		
Native cat	120	*61	*S.mississippi*	60
			S.merseyside	1
			*Ar.*61:l,v:1,5,7:(z_{57})	1
			S.(rough):b:1,5	1
Pademelon	31	0		
Potoroo	2	0		
Ring-tailed possum	2	0		
Rufus wallaby	33	0		
Tasmanian devil	37	*21	*S.mississippi*	10
			*Ar.*61:i:z	3
			S.victoria	3
			*Ar.*61:l,v:1,5,7:(z_{57})	2
			S.adelaide	2
			S.houten	2
			*Ar.*61:i:z	2
			S.wandsbek	1
			S.muenchen	1
			S.merseyside	1
			Ar.(6),14:z_{10}:z_{53}	# 1
Tiger cat	5	*4	*S.mississippi*	2
			S.warragul	2
			S.typhimurium-44	1
			*Ar.*61:l,v:1,5,7:(z_{57})	1
Wombat	23	3	*S.mississippi*	3
Black rat	2	0		
Feral cat	2	0		
Rabbit	40	0		

The first reported isolation (worldwide) of *Ar.*(6),14:z_{10}:z_{53} was from a Tasmanian devil in this survey. This serovar was subsequently isolated from water later in the investigation.

TABLE 9 (CONTINUED).

SPECIES	ANIMALS TESTED	NUMBER POSITIVE	SEROVAR	NUMBER ISOLATED
BIRDS				
Duck	5	0		
Honeyeater	1	0		
Kookaburra	1	1	*S.victoria*	1
Magpie	1	0		
Native hen	25	0		
Raven	1	0		
Silver gull	12	0		
Tawny frogmouth	1	1	*S.victoria*	1
AMPHIBIANS				
Southern toadlet	2	0		
Tree frog	4	0		
Eastern froglet	6	0		
REPTILES				
Bluetongue lizard	2	2	*S.mobeni*	1
			S.(II).43:d:z_{42}	1
Metallic skink	34	*21	*S.mississippi*	21
			S.victoria	1
Mountain dragon	1	0		
Northern snow skink	7	0		
Ocellated skink	2	0		
Slender grass skink	1	0		
Tiger snake	4	*2	*S.mississippi*	2
			*Ar.*61:i:z	1
			*Ar.*61:l,v:1,5,7:(z_{57})	1
FISH				
Short-finned eel	7	0		
Brown trout	25	0		
Blackfish	10	0		
Jollytail	6	0		
MOLLUSCS				
Garden snail	14	2	*S.victoria*	2
Oyster	**			
Mussel	**			
INSECTS				
Grasshopper	35	0		
Scarab beetle	38	0		
Corbie grub	188	0		
TOTAL Animals	350	136	isolates of 16 serovars	

* Some animals excreted multiple *Salmonella* serovars (Table 13)

** Refer to food survey for mussel and oyster results.

SURVEY OF DOMESTIC ANIMALS AND LIVESTOCK

Few of the domestic animals and livestock sampled in general surveys and listed in Table 10 carried *Salmonella*. Several animals were also screened as a consequence of *Salmonella* case investigations. These were not included in Table 10 as they were not representative of the incidence of *Salmonella* in the normal (asymptomatic) domestic animal population in Tasmania. Several domestic animals carried *Salmonella* including:

- *S.mississippi* (2 cats, 1 horse, 4 dogs)
- *S.typhimurium* (2 dogs)

TABLE 10. *SALMONELLA* ISOLATED FROM DOMESTIC ANIMALS & LIVESTOCK IN TASMANIA

SPECIES	ANIMALS TESTED	NUMBER POSITIVE	SEROVAR	NUMBER ISOLATED
Cat	2	0		
Cow	129	0		
Dog	23	0		
Emu	1	1	*S.anatum*	1
Goat	5	0		
Hen	14	0		
Horse	7	0		
Pig	5	0		
Sheep	3	0		
Wallaby	2	0		
TOTAL	190	1		

VARIATION IN THE PROPORTION OF NATIVE CATS CARRYING *SALMONELLA MISSISSIPPI*

No significant differences were observed in the carriage rate of *Salmonella mississippi* by native cats although the sample size was too small to come to any firm conclusions. However the proportion of native cats excreting *S.mississippi* at Judbury was significantly higher than the general Tasmanian native cat population.

TABLE 11. MONTHLY ISOLATIONS OF *S.MISSISSIPPI* FROM NATIVE CATS

	Judbury			Remainder of Tasmania		
MONTH	Sal (+)	Total sample	% (+)	Sal (+)	Total sample	% (+)
January	1	3				
February						
March	6	9		3	9	
April	2	6				
May	0	3		1	18	
June	7	10				
July	9	12				
August	0	1		0	2	
September	7	9		3	8	
October	7	9		1	2	
November	4	7				
December	8	11		0	2	
TOTAL	51	80	64%	8	33	20%

WATER SURVEYS

TABLE 12. *SALMONELLA* ISOLATED FROM WATER & WASTEWATER

Salmonella serovar	Reticulated & Natural	Marine & Estuarine	Sewage	Abattoir Effluent	TOTAL
*Ar.*61:l,v:1,5,7:(z_{57})	1	4			5
*Ar.*38:z_{10}:z_{53}	6	5			11
*Ar.*61:i:z	2				2
*Ar.*14:z_{10}:z_{53}	1				1
S.agona			1		1
S.anatum			1		1
S.birkenhead			3		3
S.bovismorbificans				1	1
S.bredney			1		1
S.cerro			1		1
S.chester			1		1
S.derby			2	1	3
S.give			5	1	6
S.hadar			4	5	9
S.havana			1		1
S.houten	1	1			2
S.infantis			5	1	6
S.london			2		2
S.merseyside		2			2
S.mississippi	28	18	8		54
S.muenchen				1	1
S.oranienberg			1		1
S.rowbarton		1			1
S.singapore				3	3
S.sofia		1		8	9
S.tennessee			1		1
S.typhimurium-9				2	2
S.typhimurium-12a	1			1	2
S.typhimurium-68			1		1
S.typhimurium-101		1		2	3
S.typhimurium-135			1	1	2
S.typhimurium-145		1			1
S.typhimurium-156	1				1
S.typhimurium-untyp			1		1
S.victoria	3	4			7
S.virchow				1	1
S.warragul	1	2	1		4
S.waycross			1		1
S. 9,12:-:1,5			1		1
S.(II) 16:g:-	1				1
S.(II) 16:-:-	1				1
S.(IV) 43:d:-		1			1
No. SEROVARS	12	12	21	13	43
TOTAL *SALMONELLA*	47	41	43	28	159
N^{o} SAMPLES	864	671	249	52	1847

ISOLATION OF MULTIPLE *SALMONELLA* SEROVARS

During the course of the *Salmonella* surveys several samples contained multiple *Salmonella* serovars and are shown in Table 13. The information listed in the following table is to supplement *Salmonella* surveys listed in tables 8, 9 and 12.

TABLE 13. SAMPLES CONTAINING MULTIPLE *SALMONELLA* SEROVARS

SAMPLE	*SALMONELLA* SEROVARS
Samples containing two serovars:	
brown bandicoot	*S.mississippi* & *S.warragul*
native cat	*S.mississippi* & *S.merseyside*
Tasmanian devil	*S.mississippi* & *Ar.*61:i:z
metallic skink	*S.mississippi* & *S.*victoria
Tasmanian devil	*S.adelaide* & *S.victoria*
chicken	*S.sofia* & *S.hadar*
sewage	*S.mississippi* & *S.infantis*
sea water	*S.mississippi* & *S.victoria*
poultry effluent	*S.singapore* & *S.typhimurium*-101
sea water	*S.mississippi* & *S.victoria*
poultry effluent	*S.singapore* & *S.typhimurium*-135
abattoir effluent	*S.give* & *S.bovismorbificans*
sea water	*S.mississippi* & *S.warragul*
dam water	*S.mississippi* & *S.victoria*
river water	*S.mississippi* & *S.victoria*
poultry effluent	*S.sofia* & *S.hadar*
sewage	*S.hadar* & *S.give*
poultry effluent	*S.hadar* & *S.sofia*
river water	*S.mississippi* & *S.victoria*
abattoir effluent	*S.virchow* & *S.typhimurium*-9
abattoir effluent	*S.virchow* & *S.typhimurium*-9
Samples containing three serovars:	
tiger cat	*S.mississippi*, *S.typhimurium*-44 & *Ar.*61:l,v:1,5,7:(z_{57})
tiger snake	*S.mississippi*, *Ar.*61:l,v:1,5,7:(z_{57}) & *Ar.*61:i:z
sea water	*S.mississippi*, *Ar.*38:z_{10}:z_{53} & *S.typhimurium*-101
river water	*S.mississippi*, *S.warragul* & *S.victoria*
Samples containing four serovars:	
Tasmanian devil	*S.mississippi*, *S.victoria*, *S.wandsbek* & *S.houten*

ANTIBIOTIC SENSITIVITIES OF SELECTED *SALMONELLA* ISOLATES

Salmonella mississippi isolated from native animals were all sensitive to the full range of antibiotics tested.

Salmonella hadar isolated from poultry were all resistant to streptomycin.

Salmonella sofia isolated from chicken and poultry effluent were all resistant to streptomycin.

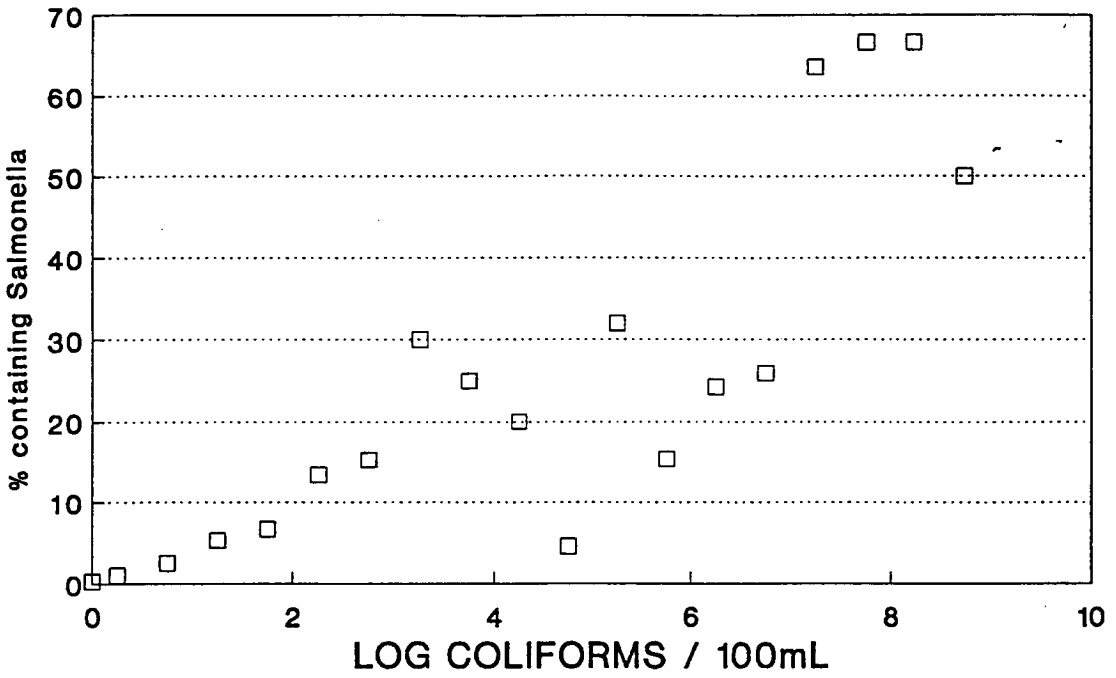
DISTRIBUTION OF *SALMONELLA* SEROVARS IN TASMANIA

The occurrence of *Salmonella* serovars from various specimen types in Tasmania is shown in Table 15. Although there were no significant two-way correlations between groups of serovars isolated from pairs of sample types it is interesting to note that all *Salmonella* serovars isolated from sea water were also found in wild animals. The only serovars isolated from all types of specimens were *S.typhimurium* and *S.mississippi*.

ENUMERATION OF INDICATOR ORGANISMS IN WATER

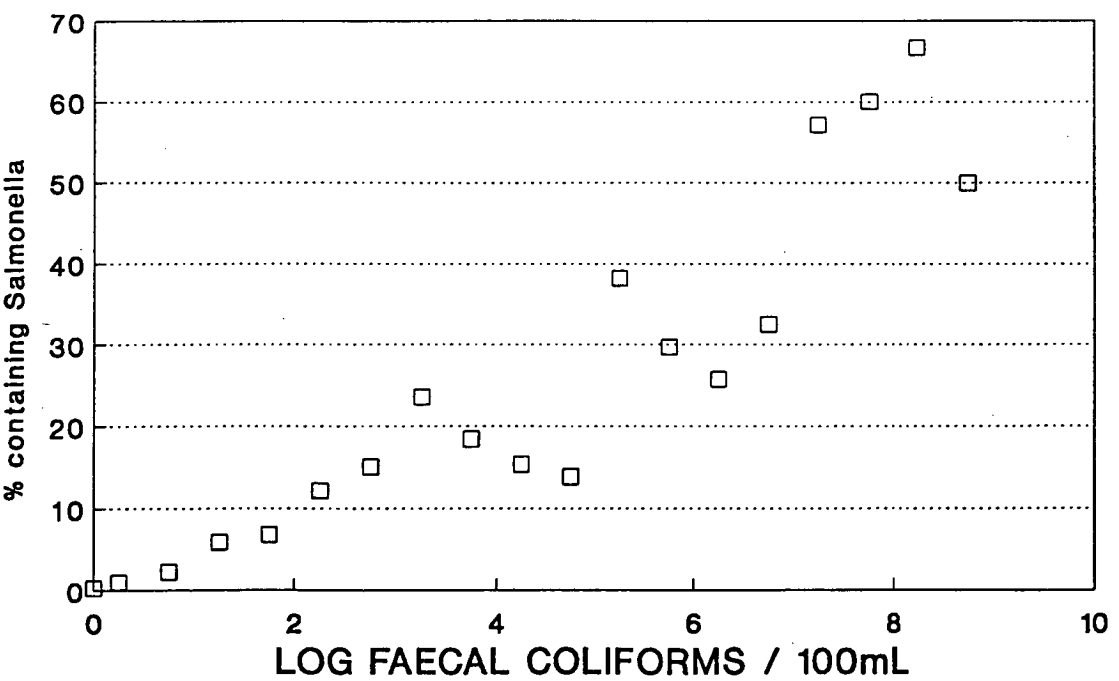
The correlation between the indicator bacteria; coliforms, faecal coliforms and faecal streptococci and *Salmonella* in water samples was examined. The information in Table 16 was collected from all water types and was used to plot graphs (Figures 2, 3 and 4) in an attempt to find a relationship between the level of indicator bacteria and the presence of *Salmonella* in water. However, each graph displayed a discontinuity at the point where effluent data became predominant which suggested the relationship differed for various water types. To investigate this possibility the data was sorted into water types in Tables 17 - 19 and replotted on Figures 26 - 28.

FIG. 2 RELATIONSHIP BETWEEN *SALMONELLA* AND COLIFORMS IN WATER



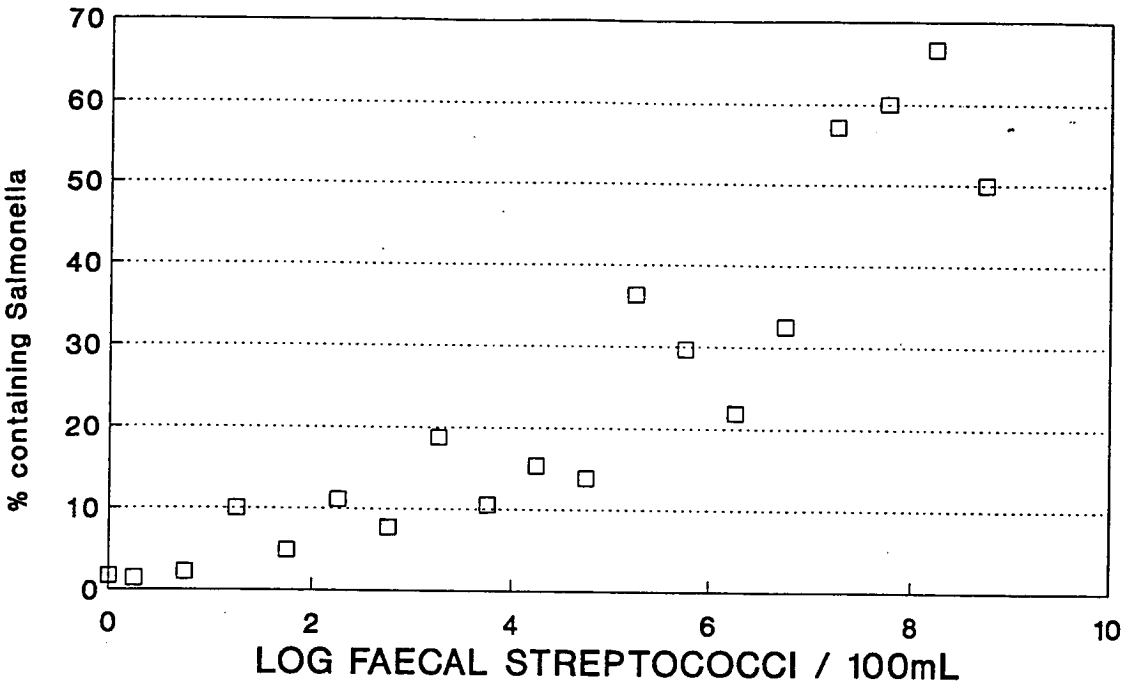
Data from all water types

FIG. 3 RELATIONSHIP BETWEEN *SALMONELLA* AND FAECAL COLIFORMS IN WATER



Data from all water types

FIG. 4 RELATIONSHIP BETWEEN *SALMONELLA* AND FAECAL STREPTOCOCCI IN WATER



Data from all water types

ENUMERATION OF *SALMONELLA* AND FAECAL COLIFORMS IN FAECES

The *Salmonella* and faecal coliform content of faeces of metallic skinks known to be excreting *S.mississippi* was determined in an assessment of possible reasons causing disparity between *Salmonella* : faecal coliform ratios of water and wastewater.

TABLE 14. *SALMONELLA* AND FAECAL COLIFORM CONTENT OF SKINK FAECES

Salmonella (cfu/g)	Faecal coliforms (cfu/g)
6.0×10^8	$<10^4$
1.1×10^9	$<10^4$
1.0×10^9	$<10^4$
6.9×10^8	$<10^4$

TABLE 15. ISOLATION OF *SALMONELLA* SEROVARS FROM VARIOUS SPECIMEN TYPES IN TASMANIA

SALMONELLA SEROVAR	Specimen type						
	H	N	S	A	D	F	M
aberdeen	+						
abony	+						
adelaide	+					+	
agona	+		+				
anatum	+		+	+	+		
*Ar.*61:l,v:1,5,7,(z_{57})	+	+				+	+
*Ar.*61:-:-	+						
*Ar.*61:i:z		+				+	
Ar.(6),14:z_{10}:z_{53}		+				+	
*Ar.*38:z10:z_{53}		+			+	+	+
*Ar.*48:i:z:Z_{57}						+	
*Ar.*61:1,5,(7)						+	
bahrenfeld	+						
bareilly	+						
birkenhead	+		+		+		
blockey	+						
bovismorbificans	+			+	+	+	
braenderup					+		
bredeney	+		+	+			
cerro	+		+				
chester	+		+		+		
cholearsuis					+		
derby	+		+	+	+		
duesseldorf	+						
eastbourne	+				+	+	
enteritidis	+					+	
give	+		+	+	+	+	
hadar	+		+	+	+		
havana	+		+				
heidelberg	+						
houten		+				+	+
infantis	+		+	+	+	+	
Java	+						
javiana	+						
johannesburg	+						
kentucky	+						
kottbus	+					+	
krefeld	+						
litchfield	+				+		
livingstone	+						
london			+				
manhattan	+						
mbdandaka	+						

TABLE 15 (CONTINUED)

SALMONELLA SEROVAR	Specimen type H	N	S	A	D	F	M
meleagridis	+						
merseyside	+					+	+
mississippi	+	+	+	+	+	+	+
mobeni						+	
montevideo	+						
muenchen	+			+	+	+	
muenster	+						
newport	+						
ohio	+						
oranienberg	+		+		+	+	
paratyphi A	+						
paratyphi B	+						
potsdam	+				+		
rowbarton		+				+	+
saintpaul	+		+		+		
senftenberg				+			
seremban	+						
singapore	+		+	+			
sofia	+			+	+	+	+
stanley	+						
taksony	+						
tennessee	+		+			+	
thompson	+						
typhi	+						
typhimurium	+	+	+	+	+	+	+
victoria	+	+			+	+	+
virchow	+			+			
wandsbek						+	
warragul	+	+				+	+
waycross	+		+				
zanzibar					+		
I.4,12:d:-				+	+		
I.9,12:-:1,5			+				
II.43:d:z_{42}		+				+	
II.16:g:-		+					
II.16:-:-		+					
IV.43:d:-		+				+	+
(rough):b:1,5	+					+	
IV.(rough):43:-						+	

SPECIMEN TYPES:

H	human
N	reticulated & natural fresh water
S	sewage effluent
A	abattoirs / effluent
D	domestic & farm animals
F	indigenous fauna
M	sea water

TABLE 16. COMPARISON OF INDICATOR BACTERIA LEVELS AND PRESENCE OF *SALMONELLA* IN WATERS

Indicator organism /100mL	COLIFORMS		FAECAL COLIFORMS		FAECAL STREPTOCOCCI	
	Sample	Sal (+)	Sample	Sal (+)	Sample	Sal (+)
0	706	2	707	2	60	1
10^0-$10^{0.5}$	201	2	205	2	71	1
$10^{0.5}$-10^1	199	5	219	5	91	2
10^1-$10^{1.5}$	114	6	119	7	40	4
$10^{1.5}$-10^2	137	9	148	10	82	4
10^2-$10^{2.5}$	67	9	82	10	54	6
$10^{2.5}$-10^3	59	9	73	11	52	4
10^3-$10^{3.5}$	40	12	51	4	32	6
$10^{3.5}$-10^4	16	4	38	4	38	4
10^4-$10^{4.5}$	15	1	26	2	26	4
$10^{4.5}$-10^5	22	1	36	5	36	5
10^5-$10^{5.5}$	25	8	34	13	33	12
$10^{5.5}$-10^6	26	4	37	11	37	11
10^6-$10^{6.5}$	33	8	35	9	32	7
$10^{6.5}$-10^7	27	7	40	13	40	13
10^7-$10^{7.5}$	11	7	21	12	21	12
$10^{7.5}$-10^8	3	2	5	3	5	3
10^8-$10^{8.5}$	3	2	3	2	3	2
$10^{8.5}$-10^9	2	1	2	1	2	1
TOTAL	1706	99	1881	126	755	102

TABLE 17. COMPARISON OF INDICATOR BACTERIA LEVELS AND PRESENCE OF *SALMONELLA* IN FRESH WATER

Indicator organism /100mL	COLIFORMS		FAECAL COLIFORMS		FAECAL STREPTOCOCCI	
	Sample	Sal (+)	Sample	Sal (+)	Sample	Sal (+)
0	430	2	430	2	47	1
10^0-$10^{0.5}$	123	2	126	2	61	1
$10^{0.5}$-10^1	100	4	105	4	69	2
10^1-$10^{1.5}$	58	4	61	5	29	4
$10^{1.5}$-10^2	58	4	66	5	50	4
10^2-$10^{2.5}$	30	4	36	4	29	4
$10^{2.5}$-10^3	22	2	28	3	24	3
10^3-$10^{3.5}$	11	3	15	3	12	3
$10^{3.5}$-10^4	6	4	7	4	7	4
TOTAL	838	29	874	32	328	26

TABLE 18. COMPARISON OF INDICATOR BACTERIA LEVELS AND PRESENCE OF SALMONELLA IN SEA WATER

Indicator organism /100mL	COLIFORMS		FAECAL COLIFORMS		FAECAL STREPTOCOCCI	
	Sample	Sal (+)	Sample	Sal (+)	Sample	Sal (+)
0	273	0	273	0	9	0
10^0-$10^{0.5}$	75	0	75	0	6	0
$10^{0.5}$-10^1	95	1	108	1	16	0
10^1-$10^{1.5}$	53	2	54	2	7	0
$10^{1.5}$-10^2	65	5	66	5	16	0
10^2-$10^{2.5}$	31	5	34	6	14	2
$10^{2.5}$-10^3	26	7	27	8	10	1
10^3-$10^{3.5}$	23	9	24	9	8	3
$10^{3.5}$-10^4	2	0	2	0	2	0
10^4-$10^{4.5}$	3	2	4	3	4	3
$10^{4.5}$-10^5	2	1	2	1	2	1
10^5-$10^{5.5}$	2	2	2	2	1	1
TOTAL	650	34	671	37	95	11

TABLE 19. COMPARISON OF INDICATOR BACTERIA LEVELS AND PRESENCE OF *SALMONELLA* IN WASTEWATER

Indicator organism /100mL	COLIFORMS		FAECAL COLIFORMS		FAECAL STREPTOCOCCI	
	Sample	Sal (+)	Sample	Sal (+)	Sample	Sal (+)
0	3	0	4	0	4	0
10^0-$10^{0.5}$	3	0	4	0	4	0
$10^{0.5}$-10^1	4	0	6	0	6	0
10^1-$10^{1.5}$	3	0	4	0	4	0
$10^{1.5}$-10^2	13	0	16	0	16	0
10^2-$10^{2.5}$	6	0	12	0	11	0
$10^{2.5}$-10^3	11	0	18	0	18	0
10^3-$10^{3.5}$	6	0	12	0	12	0
$10^{3.5}$-10^4	8	0	29	0	29	0
10^4-$10^{4.5}$	12	1	22	1	22	1
$10^{4.5}$-10^5	20	0	34	4	34	4
10^5-$10^{5.5}$	23	6	32	11	32	11
$10^{5.5}$-10^6	26	4	37	11	37	11
10^6-$10^{6.5}$	33	8	35	9	32	7
$10^{6.5}$-10^7	27	7	40	13	40	13
10^7-$10^{7.5}$	11	7	21	12	21	12
$10^{7.5}$-10^8	3	2	5	3	5	3
10^8-$10^{8.5}$	3	2	3	2	3	2
$10^{8.5}$-10^9	2	1	2	1	2	1
TOTAL	217	38	336	67	332	65

TABLE 20 MONTHLY VARIATION OF WATER BORNE *SALMONELLA*

RETICULATED WATER

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Nº. samples tested	57	24	52	77	87	67	84	45	11	10	13	29	556
S.mississippi	1	0	2	0	1	0	0	0	0	0	1	1	6
other *Salmonella* spp.	1	0	0	0	1	1	0	0	0	0	0	0	3
Total *Salmonella* (+)	2	0	2	0	2	1	0	0	0	0	1	1	9

NATURAL WATERS

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Nº. samples tested	37	20	28	38	59	39	27	12	14	15	9	21	319
S.mississippi	4	3	3	1	0	0	0	0	1	0	1	2	15
other *Salmonella* spp.	2	0	1	0	1	1	0	0	0	0	0	0	5
S.mississippi & *Salmonella* sp.	1	0	0	0	0	0	0	0	0	0	0	2	3
Total *Salmonella* (+)	7	3	4	1	1	1	0	0	1	0	1	4	23

MARINE & ESTUARINE WATERS

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Nº. samples tested	61	45	79	18	45	53	55	57	57	59	80	71	680
S.mississippi	0	1	1	0	0	1	1	0	0	1	3	6	14
other *Salmonella* spp.	4	0	5	0	0	0	0	1	1	0	1	6	18
S.mississippi & *Salmonella* sp.	3	0	2	0	0	0	0	0	0	0	0	0	5
Total *Salmonella* (+)	7	1	8	0	0	1	1	1	1	1	4	12	37

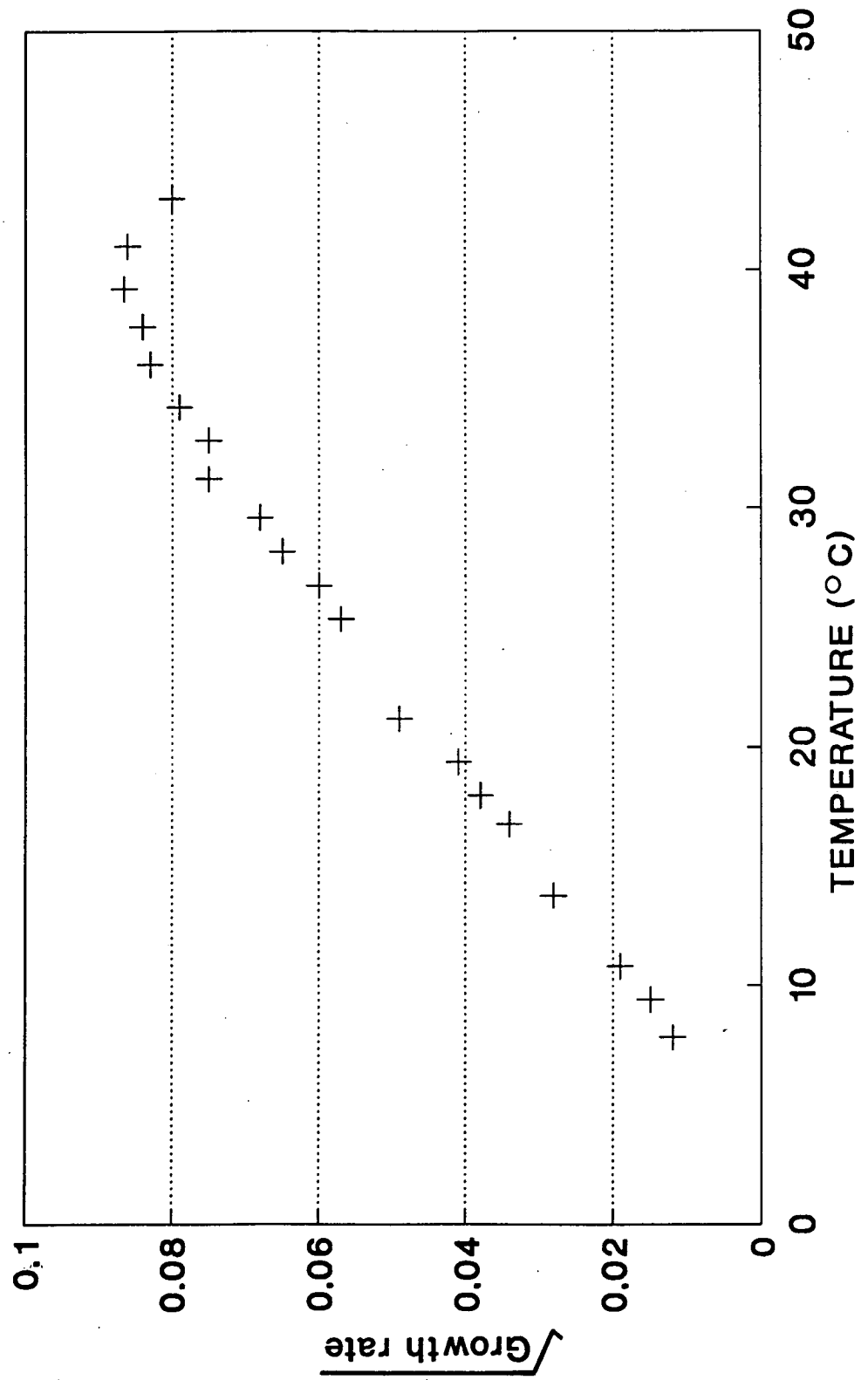
TABLE 20 (CONT'D). MONTHLY VARIATION OF WATER BORNE *SALMONELLA*

SEWAGE	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
N°. samples tested	16	18	20	14	14	35	16	29	21	18	37	11	249
S.mississippi	1	0	2	2	0	0	0	0	0	0	2	0	7
other *Salmonella* spp.	3	0	5	5	3	3	1	2	1	6	2	2	33
S.mississippi & *Salmonella* sp.	0	0	0	1	0	0	0	0	0	0	0	0	1
Total *Salmonella* (+)	4	0	7	8	3	3	1	2	1	6	4	2	41
ABATTOIR EFFLUENT	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
N°. samples tested	8	7	8	5	2	0	5	5	1	6	2	3	52
Total *Salmonella* (+) *	4	1	5	3	1	0	5	1	1	0	0	1	22

* No *S.mississippi* were isolated from abattior effluent in this survey.

The T_{min} of 3.2°C- 3.4°C was derived from this data.

FIG. 5 GROWTH TEMPERATURE CHARACTERISTIC OF *S. MISSISSIPPI*



The growth temperature curve (Fig.5) gave T_{min}, T_{opt} and T_{max} growth temperatures of 3.20-3.4°C, 39.7°C and 48.0°C, respectively, for *S.mississippi*. Observed minimum growth temperatures determined for several strains of *Salmonella* fell between 5.5°C and 7.0°C. Comparisons between minimum observed growth temperatures for various strains of *S.mississippi* and other *Salmonella* serovars are shown in Table 21.

TABLE 21. GROWTH OF *SALMONELLAE* AT CHILL TEMPERATURES

Salmonella serovar	Strain	Incubation temperature (°C) 7.0	6.5	6.0	5.5
S.mississippi	M9	+	+	-	-
S.mississippi	M28	+	+	-	-
S.mississippi	M40	+	+	-	-
S.mississippi	M50	+	+	+	-
S.mississippi	M59	+	-	-	-
S.mississippi	M60	+	+	-	-
S.mississippi	M189	+	+	-	-
S.mississippi	M210	+	+	-	-
S.agona	87J9747	+	-	-	-
S.bovismorbificans	85J2812	+	-	-	-
S.derby	86J2776	+	-	-	-
S.merseyside	87J4180	+	+	-	-
S.muenchen	87J6769	+	+	-	-
S.oranienberg	87J8467	+	-	-	-
S.typhimurium-9	RobDog	+	-	-	-
S.typhimurium-135	89J2588	+	-	-	-
S.warragul	89J3696	+	+	-	-
Ar.(6),14:z_{10}:z_{53}	88J4834	+	+	+	-

SURVIVAL OF *SALMONELLA* IN SOIL

Survival of *Salmonella* in the natural environment was examined by measurement of the persistence of four *Salmonella* serovars after inoculation on clay loams from three different locations. No significant differences of survival period were observed in the soils tested although recovery was sporadic from some trials.

TABLE 22 RECOVERY OF *SALMONELLA* FROM INOCULATED SOILS

	S.typhimurium			*S.mississippi*			*S.sofia*			*S.derby*		
SOIL WEEK	A	B	C	A	B	C	A	B	C	A	B	C
2	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	-	-	-	+	-	-
4	+	-	-	+	+	+	-	+	+	-	-	-
5	-	-	-	-	+	-	-	-	-	-	-	+
6	+	-	+	-	-	-	-	-	-	+	+	-
7	-	-	+	-	+	-	-	+	+	-	-	-
8	-	-	-	-	-	-	+	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	+	-	-	-	-	-	-
11	+	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-

Soil A Clay loam (Judbury pasture)
Soil B Clay loam (Lenah Valley lawn)
Soil C Clay loam (Sorell pasture)

Survival was measured in terms of decimal reduction times, or the number of days required for a tenfold decrease to occur in the number of culturable bacteria. Decimal reduction times were calculated for several isolates to determine whether *S.mississippi* differed greatly from other salmonellae reported in the literature.

TABLE 23. SURVIVAL OF *SALMONELLA MISSISSIPPI* IN STERILE WATER

Culture	Decimal reduction time (days) 30°C	15°C	4°C
M9	1.3	3.0	11
M28	1.5	3.5	11
M40	1.6	4.2	15
M50	1.8	5.8	21
M59	1.1	3.6	11
M60	1.2	4.9	6.7
M189	1.7	5.3	14
M210	1.6	3.4	7.6
MEAN	1.5	4.2	12
S.D.	2.5	1.0	4.5

TABLE 24. SURVIVAL OF *SALMONELLA MISSISSIPPI* IN STERILE SEA WATER

Culture	Decimal reduction time (days) 30°C	15°C	4°C
M9	1.4	3.8	4.4
M28	1.2	2.6	3.6
M40	1.3	3.0	4.6
M50	1.9	6.0	4.9
M59	1.7	5.6	5.8
M60	1.6	3.2	3.6
M189	1.2	2.3	5.3
M210	1.5	4.1	3.4
MEAN	1.5	3.8	4.2
S.D.	2.5	1.4	0.9

THE EFFECT OF SUNLIGHT ON THE SURVIVAL OF *SALMONELLA* & *E.COLI*

There was wide variation in survival rates among the 31 aqueous suspensions of *S.mississippi*, other *Salmonella* serovars and *E.coli* (shown in Table 25) after a full day's exposure of the bacteria to sunlight. No significant differences were observed but the range of survival rates from the nine *S.mississippi* strains was more restricted than that of the ten other *Salmonella* serovars used.

As there was obvious variation within strains the experiment was repeated with six replicates of certain bacteria to more accurately assess differences between their respective tolerances to sunlight. The experiment attempted to mimic natural conditions as much as possible so sunlight was used in preference to a more reproducible light source which does not give comparable results to sunlight (Davies & Evison, 1991). The results presented in Tables 26, 27 and 28 were from three separate days and therefore not directly comparable. However, on the assumptions that the incident sunlight was similar on all three days (all of which were mainly overcast and spanned a three week period) and that coliform decay rates are directly proportional to the amount of incident light (Chamberlain & Mitchell, 1975), the results may be compared by standardisation of the mean survival rate of *S.mississippi* M9 which was repeated in each trial. The relative survival rates derived are presented in Table 29.

TABLE 25. SURVIVAL OF *SALMONELLA* AND *E.COLI* IN SUNLIGHT

Strain	Culture	Log Reduction	% survival
S.warragul	89J3695	0.30	50
S.mississippi	M192S	0.43	37
S.mississippi	M93	0.53	29
S.mississippi	M93S	0.53	29
S.sofia		0.57	27
S.mississippi	M192	0.60	25
S.mississippi	M9	0.62	24
S.mississippi	M97	0.68	21
S.bovismorbificans	85J2812	0.69	20
S.mississippi	M97S	0.75	18
S.sofia	D2	0.77	17
S.mississippi	M96	0.82	15
S.mississippi	M31	0.89	13
S.typhimurium-135	N	0.93	12
S.houten	87J1404	0.93	12
S.typhimurium-135		0.93	12
S.typhimurium-9	Robdog	1.04	9.1
S.mississippi	M117	1.08	8.3
S.mississippi	M90S	1.12	7.5
S.mississippi	M90	1.19	6.5
S.derby	83J10010	1.23	5.9
S.sofia	S	1.23	5.9
S.mississippi	M91	1.23	5.8
E.coli		1.60	2.5
S.senftenberg	87J8468	1.69	2.0
S.choleraesuis	S	2.02	0.96
S.victoria	86J4141	2.07	0.85
E.coli	S	2.12	0.76
S.choleraesuis		2.21	0.62
S.typhimurium-135		2.41	0.39

TABLE 26. COMPARATIVE SURVIVAL OF *S. MISSISSIPPI* AND *S. CHOLERAESUIS* IN FRESH WATER WHEN EXPOSED TO NATURAL SUNLIGHT

Strain	Culture	Log Reduction	% survival
S.mississippi	M9	0.43	37
S.mississippi	M9	0.66	22
S.mississippi	M9	0.23	60
S.mississippi	M9	0.40	40
S.mississippi	M9	0.43	37
S.mississippi	M9	0.63	23
MEAN		0.49	37
SD		0.16	14
S.choleraesuis	RHH	1.06	8.7
S.choleraesuis	RHH	1.86	1.4
S.choleraesuis	RHH	2.03	0.93
S.choleraesuis	RHH	1.60	2.5
S.choleraesuis	RHH	2.01	0.98
S.choleraesuis	RHH	2.09	0.81
MEAN		1.88	2.6
SD		0.39	3.1

TABLE 27. COMPARATIVE SURVIVAL OF *S. MISSISSIPPI* AND *E. COLI* IN FRESH WATER WHEN EXPOSED TO NATURAL SUNLIGHT

Strain	Culture	Log Reduction	% survival
S.mississippi	M9	0.79	16
S.mississippi	M9	0.72	19
S.mississippi	M9	0.60	25
S.mississippi	M9	0.45	35
S.mississippi	M9	0.40	40
S.mississippi	M9	0.33	47
MEAN		0.58	30
SD		0.18	12
E.coli	RHH	2.04	0.90
E.coli	RHH	1.99	1.0
E.coli	RHH	1.84	1.4
E.coli	RHH	1.75	1.8
E.coli	RHH	1.64	2.3
E.coli	RHH	1.21	6.3
MEAN		1.82	2.3
SD		0.30	2.0

TABLE 28. COMPARATIVE SURVIVAL OF *SALMONELLA* AND *E.COLI* IN FRESH WATER WHEN EXPOSED TO NATURAL SUNLIGHT

Strain	Culture	Log Reduction	% survival
S.mississippi	M9	0.15	71
S.mississippi	M9	0.34	45
S.mississippi	M9	0.30	50
S.mississippi	M9	0.30	50
S.mississippi	M9	0.17	69
S.mississippi	M9	0.28	53
MEAN		0.26	56
SD		0.08	11
S.typhimurium-9	912432SD	0.27	54
S.typhimurium-9	912432SD	0.32	47
S.typhimurium-9	912432SD	0.64	23
S.typhimurium-9	912432SD	0.58	26
S.typhimurium-9	912432SD	0.54	28
S.typhimurium-9	912432SD	0.34	45
MEAN		0.47	37
SD		0.16	13
S.sofia	89F35	0.27	54
S.sofia	89F35	0.20	64
S.sofia	89F35	0.17	68
S.sofia	89F35	0.20	65
S.sofia	89F35	0.37	42
S.sofia	89F35	0.29	52
MEAN		0.25	57
SD		0.07	10
E.coli	914666	0.81	15
E.coli	914666	0.53	29
E.coli	914666	0.88	13
E.coli	914666	0.97	11
E.coli	914666	0.99	10
E.coli	914666	0.85	14
MEAN		0.86	15
SD		0.17	7

TABLE 29. RELATIVE SURVIVAL RATES OF *SALMONELLA* AND *E.COLI* IN FRESH WATER WHEN EXPOSED TO NATURAL SUNLIGHT

Strain	Culture /source	RELATIVE SURVIVAL RATE MEAN	SD
S.sofia	89F35	57	10
S.mississippi	M9	56	11
S.mississippi	M9	56	21
S.mississippi	M9	56	22
S.typhimurium-9	912432SD	37	13
E.coli	914666	15	7
E.coli	RHH	4.3	3.7
S.choleraesuis	RHH	3.9	4.7

There is considerable variation in the ability of strains of *Salmonella* and *E.coli* to survive in sunlight. Preliminary results indicate that "environmental" strains of *Salmonella* may survive better than invasive strains and *E.coli*.

PERIOD OF EXCRETION OF *SALMONELLA* IN SOME NATIVE ANIMALS

To investigate the possibility of an affinity between certain animals and *Salmonella mississippi* several animal experiments were carried out.

In the initial trial, three naturally infected native cats excreted *S.mississippi* for the duration of their 11-16 week captivity (Table 30). Such a long period of excretion without any sign of apparent symptoms may signify that native cats become carriers rather than continually being reinfected via contaminated food or water. To test the hypothesis that an affinity exists between *S.mississippi* and certain animals, a further two trials were carried out using native cats and brush possums inoculated with *S.mississippi* and three other *Salmonella* serovars.

Salmonella.mississippi, *S.muenchen*, *S.typhimurium* and *S.victoria* were each fed to pairs of native cats. Three of the animals showed some mild diarrhoea for a few days immediately after being inoculated but otherwise appeared to be in good health. The inoculated strains persisted for between one and six weeks, significantly less than that displayed by *S.mississippi* which was still being excreted after 13 weeks (Table 31).

Six brush possums were inoculated with *S.mississippi* and three other *Salmonella* strains. Of the three possums inoculated with *S.mississippi*, two ceased to excrete the organism after two weeks and the other after six weeks. Those infected with *S.muenchen* and *S.victoria* ceased excreting after one and five weeks respectively. *S.typhimurium* was still present in scats after 12 weeks but not 14 weeks (Table 32).

TABLE 30. PERSISTENCE OF *SALMONELLA MISSISSIPPI* IN NATIVE CATS

WEEK	REPLICATE A	B	C	D	E
0	+	+	+	+	+
1	-	+	+	.	.
2	-	+	-	+	+
3	+	+	-	.	.
4	-	+	+	+	+
5	+	+	+	+	+
6	-	+	+	+	+
7	+	+	+	+	+
8	+	+	.	+	+
9	+	+	-	+	+
10	.	.	+	+	+
11	+	+	-	+	+
12	r	r	r	+	+
13				+	+
14				+	-
16				+	-

. denotes not tested
r denotes animal released

TABLE 31. PERSISTENCE OF SALMONELLAE IN NATIVE CATS

SEROVAR	*S.typhimurium*		*S.muenchen*		*S.victoria*		*S.mississippi*	
Replicate WEEK	A	B	A	B	A	B	A	B
0	+	+	+	+	+	+	+	+
1	+	-	+	+	-	+	+	+
2	+	-	+	-	-	+	+	+
3	+	-	.	+	-	-	+	+
4	-	-	+	+	-	-	+	+
5	+	-	d	-	-	-	+	+
6	+	-		-	-	-	+	+
7	-	-		-	-	-	+	+
8	-	-		-	-	-	+	+
9	-	-		-	-	-	+	+
10	-	-		-	-	-	+	+
11	-	-		-	-	-	+	+
12	r	r		r	r	r	+	+
13							+	+

d denotes animal died (malignant growth on heart)
. denotes not tested
r denotes animal released

TABLE 32 PERSISTENCE OF SALMONELLAE IN BRUSHTAIL POSSUMS

DAY	*S.mississippi* 1	2	3	*S.victoria* 1	*S.typhimurium* 1	*S.muenchen* 1
2	.	-	+	+	-	+
3	.	+	+	+	-	+
4	-	+	+	+	-	+
6	+	+	+	+	+	+
14	+	+	+	+	+	-
16	-	.	-	+	+	-
20	-	-	-	-	+	-
28	-	-	-	+	+	-
30	-	-	-	-	+	-
34	-	+	-	+	+	-
42	-	+	-	-	+	-
49	-	-	-	-	+	-
55	-	-	-	-	-	-
64	-	-	-	-	-	-
73	-	-	-	-	-	-
83	-	-	.	-	+	-
96	r	r	r	r	-	r

. denotes not tested
r denotes animal released

DISCUSSION

INTRODUCTION

Several aspects of the incidence of enteric notifiable diseases, particularly salmonellosis, in Tasmania will be addressed in the following discussion.

The effectiveness of the Tasmanian communicable disease notification system, which is fundamental to the success of epidemiological studies is considered. A comparison of the frequency of human salmonellosis at state, national and international levels is examined, followed by a detailed examination of the *Salmonella* serovars encountered in Tasmania.

The epidemiology of several *Salmonella* serovars will be discussed, with particular emphasis on *S.mississippi*, which is unique in being encountered regularly in Tasmania but only spasmodically in mainland Australia and other geographic locations of the world. The epidemiological investigation of *S.mississippi* was primarily to identify a reservoir or vehicle of infection. As a secondary consideration some preliminary trials were carried out to investigate its persistence in the environment.

The significance of *Salmonella* in water and the effectiveness of indicator bacteria to monitor water quality will be discussed.

COMMUNICABLE DISEASE REPORTING SCHEME

Early Tasmanian *Salmonella* data is not reliable. For example 88 Tasmanian isolates were serotyped at the Institute of Medical and Veterinary Science (IVMS) in 1976 but only 30 *Salmonella* notifications were received by the Department of Health during the same period. However, discrepancies of such magnitude no longer occur and the small variations currently seen may be due to failure to edit duplication.

To investigate *Salmonella* epidemiology in Tasmania an adequate reporting scheme needed to be devised. The previous system of medical practitioner notifications was sporadic even though there is a legal requirement to notify the Tasmanian Department of Health. Notifications by pathology laboratories were not made until serovar identification was confirmed by the *Salmonella*

reference laboratories at the IVMS or the Melbourne Diagnostic Unit (MDU). This entailed a minimum delay of two weeks between isolation and investigation by which time epidemiological investigation was ineffective.

To reduce the reporting time several suggestions were adopted. The pathology laboratories were to forward *Salmonella* notifications as soon as they were apparent, noting the results of any serology performed. This measure reduced the notification time by 10-20 days and provided a preliminary indication of the serovar. If an outbreak investigation was in progress the culture could be obtained for further analysis by the State Microbiology Laboratory. Also a copy of the reference laboratory report was sent directly to the Director of Public Health instead of via the forwarding pathology laboratory. This reduced the delay by a further 2-7 days. These improvements to the notification system enabled effective epidemiological investigation.

On a national level there were also improvements which made the epidemiological investigation of salmonellosis more effective. In 1980 the National Salmonella Surveillance Scheme (NSSS) was instituted in an attempt to centralise data hitherto held by separate laboratories throughout Australia. It now provides fairly comprehensive information on enteric diseases at a national level and is a very useful epidemiological tool.

HUMAN ENTERIC BACTERIAL DISEASES IN AUSTRALIA

An indication of the relative importance of notifiable enteric bacterial diseases in Australia can be seen on examination of NSSS data. For the period 1987-9 the average case rates per 100,000 population were: *Salmonella*, 31; *Campylobacter*, 16; *Shigella*, 4.7; enteropathogenic *E.coli*, 0.14; *Vibrio*, 0.02. Of these organisms only *Salmonella* and *Shigella* will be considered as only these are notifiable in all Australian states.

Case rates of *Shigella* are quite variable between states (NSSS). Tasmania and the Australian Capital Territory share the lowest rate of 0.8 / 10^5 population, followed by New South Wales, Victoria, Queensland and South Australia with between 1.6 and 3.2. Western Australia and the Northern Territory have much higher incidence of shigellosis with case rates of 20 and 120 / 10^5 respectively. The high occurrence in the latter two states is a reflection of the prevalence of shigellosis in the Aboriginal population (NSSS).

The frequency of human salmonellosis in Australia is quite varied between states and territories, as shown by the following table.

TABLE 33. RATES OF HUMAN SALMONELLOSIS IN AUSTRALIAN STATES (CASE RATES PER 100,000 POPULATION)

YEAR	ACT	NSW	Vic	Qld	SA	WA	Tas	NT	Aust
1985	55.3	21.4	12.0	43.1	28.6	59.7	18.5	312	28.6
1986	19.2	17.1	12.7	50.4	25.6	52.9	13.3	265	27.6
1987	21.4	16.0	12.3	52.4	23.2	50.2	28.2	237	28.3
1988	21.4	19.6	18.0	62.6	25.8	53.0	25.4	227	33.5
1989	32.1	24.4	26.8	56.5	35.1	49.8	38.7	269	36.2
1990	20.0	24.7	20.0	59.5	39.5	46.2	30.5	235	31.6
MEAN	28.2	20.5	17.0	54.1	29.6	52.0	28.5	258	31.0
SD	14.1	3.6	5.9	7.0	6.3	4.5	9.0	32	3.4

Data supplied courtesy of the NSSS

The extremely high incidence of human salmonellosis was seen mainly in the sparsely populated regions of northern Australia whereas the lowest rates occurred in the most populated states.

EPIDEMIOLOGY OF *SALMONELLA* IN TASMANIA

Cases of non-human *Salmonella* are not included as surveys are carried out for a variety of reasons and frequently omit some areas thereby causing a biased pattern of distribution. Such bias is eliminated from data from human sources if all salmonellosis cases were notified as required by statute.

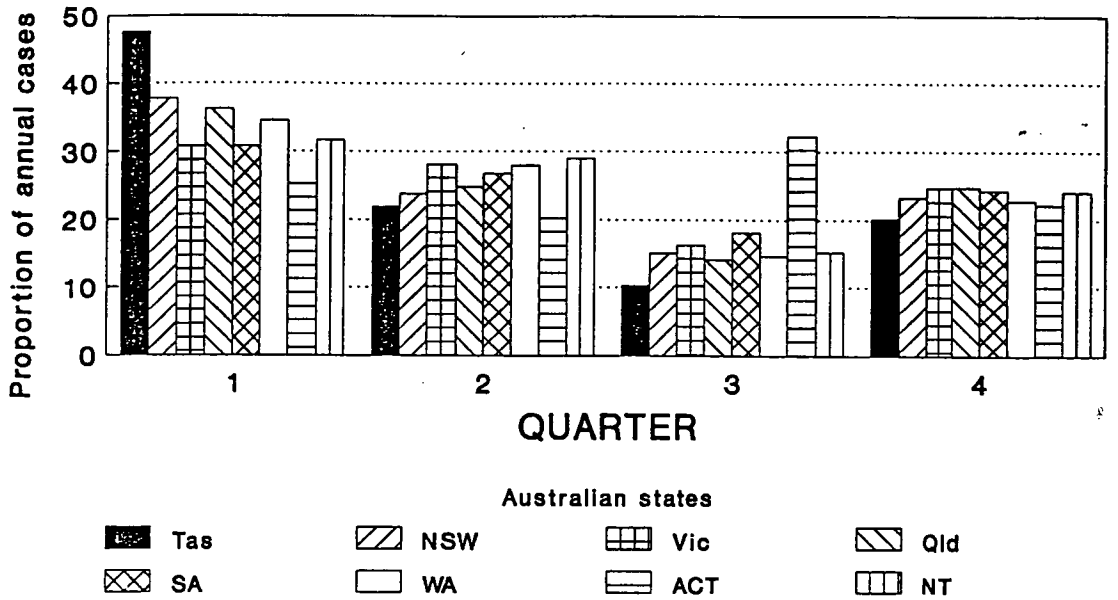
Annual rates of human salmonellosis / 100,000 population are shown on Table 7. The mean annual incidence in Tasmania is $17.3/10^5$ (SD = 9.7). This rate is lower than $25.8/10^5$ recorded on Table 33 because it is derived from corrected data from which duplications were removed and in which familial outbreaks were reduced to single cases. The distribution pattern of human *Salmonella* in Tasmania was generally commensurate with population density except on King and Flinders Islands where the incidence of salmonellosis was significantly higher per head of population than for the remainder of Tasmania.

SEASONAL VARIATION

Throughout Australia human salmonellosis peaks in the January to March quarter. The lowest occurrences are between July and September. The increased incidence of human salmonellosis during summer has also been described in other parts of the world (Sanders *et al.*, 1965; Hobbs & Gilbert, 1978; Ram *et al.*, 1987; Skirrow, 1987; CDC, 1990). In the USA the broad summer and autumn peak (Sanders *et al.*, 1965) followed the same pattern as food poisoning outbreaks (Bean & Griffin, 1990). In the UK the sharp rise in salmonellosis in early summer is maintained until autumn (Skirrow, 1987). The pattern in India is characterised by two peaks (Ram *et al.*, 1987). The first occurs in early summer and the second coincides with the onset of the hot rainy season in autumn.

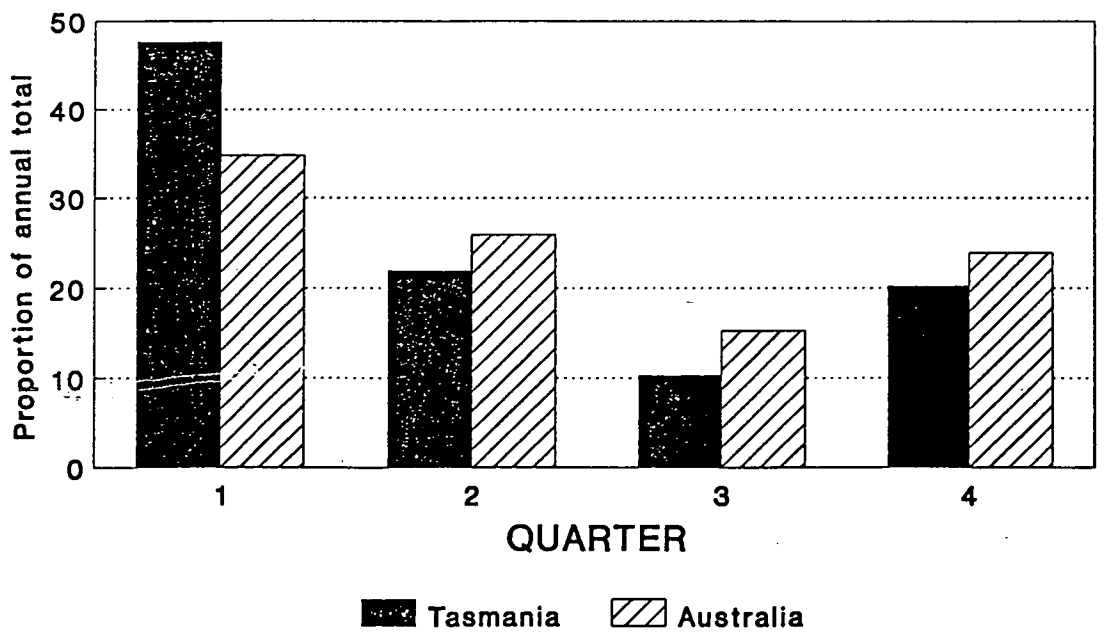
However, the seasonal difference was more pronounced in Tasmania than any other state (Fig.6), with almost half the cases occurring in the first quarter compared with a third for the remaining states (Fig.7). This phenomenon is consistent, the pattern being recurrent over a number of years (Fig.9). The seasonal variation in human salmonellosis between Tasmania and Australia is due to *S.mississippi*. This can be seen from the close resemblance between the seasonal distribution of salmonellosis in Tasmania and mainland Australia on omission of *S.mississippi* from the Tasmanian data (Fig.8). The increased summer peak and winter trough observed for *S.mississippi* differ significantly ($P<0.001$) from other *Salmonella* serovars in Tasmania.

FIG. 6 SEASONAL VARIATION OF HUMAN SALMONELLOSIS IN AUSTRALIAN STATES



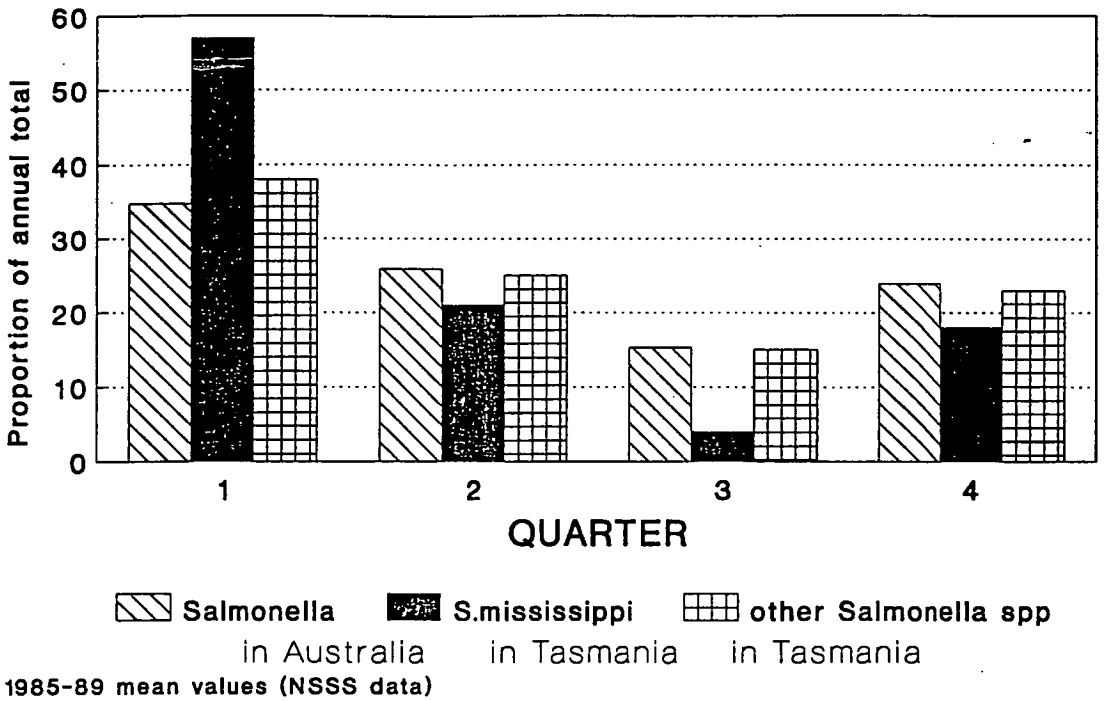
1985-89 mean values (NSSS data)

FIG. 7 SEASONAL VARIATION IN HUMAN SALMONELLOSIS: QUARTERLY AVERAGES



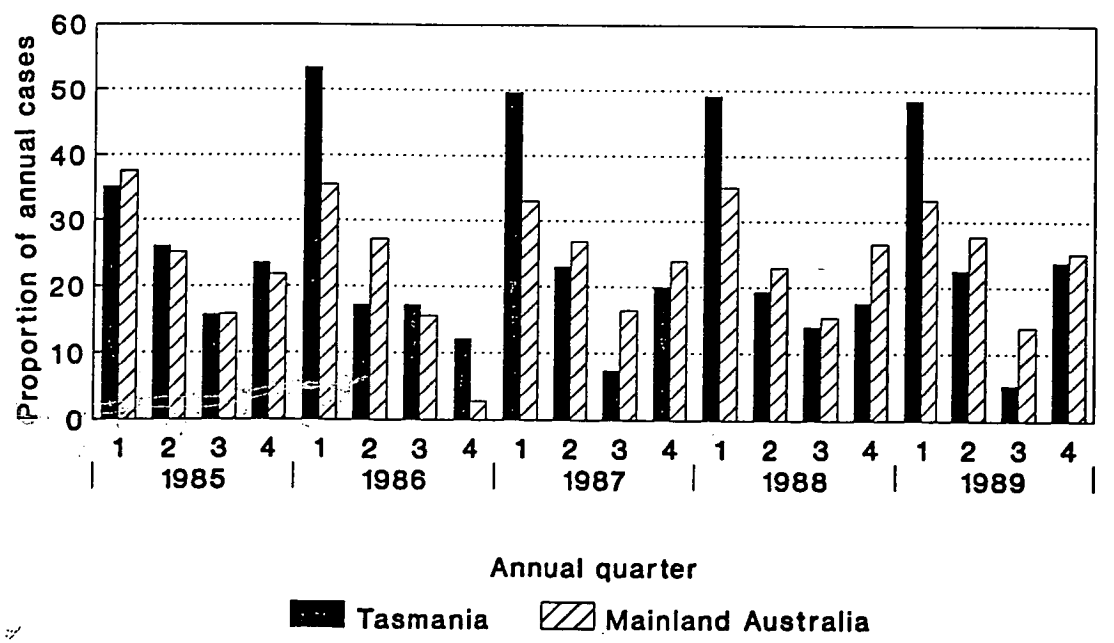
1985-89 mean values (NSSS data)

FIG. 8 SEASONAL VARIATION IN HUMAN SALMONELLOSIS: QUARTERLY AVERAGES AND SEROVAR DIFFERENCES



1985-89 mean values (NSSS data)

FIG. 9 SEASONAL VARIATION IN HUMAN SALMONELLOSIS IN TASMANIA AND MAINLAND AUSTRALIA



In the period 1984-1991, three of 149 patients with diarrhoea who had no known association with *Salmonella* were found to be excreting the pathogen. The isolation rate of 2% was comparable to similar rates found throughout Tasmania. At the major hospital in Hobart, *Salmonella* was isolated from stool samples of inpatients at a rate of between 0.33 and 0.92% (mean 0.6%) during the period 1984-90. At the equivalent infirmary in Launceston the range was slightly higher, 1.1-2.2% (mean 1.8%) in the period 1988-1990. Rates of 2.7% and 2.1% were noted from the private pathology laboratories on the northwestern and northern regions of Tasmania respectively during 1990. In southern Tasmania a local study isolated six *Salmonella* from 201 stool specimens of diarrhoea patients with an isolation rate of 3% (Hunt, 1989). In comparison, studies of diarrhoea patients in other parts of the world have revealed *Salmonella* isolation rates of between 1.2 and 12% (Kourany & Telford, 1981; Skirrow, 1987; Kain *et al.*, 1991). As would be expected, higher incidence is generally found in less developed countries. These results seem to indicate the proportion of gastroenteritis due to *Salmonella* in Tasmania is comparable with that of other developed countries.

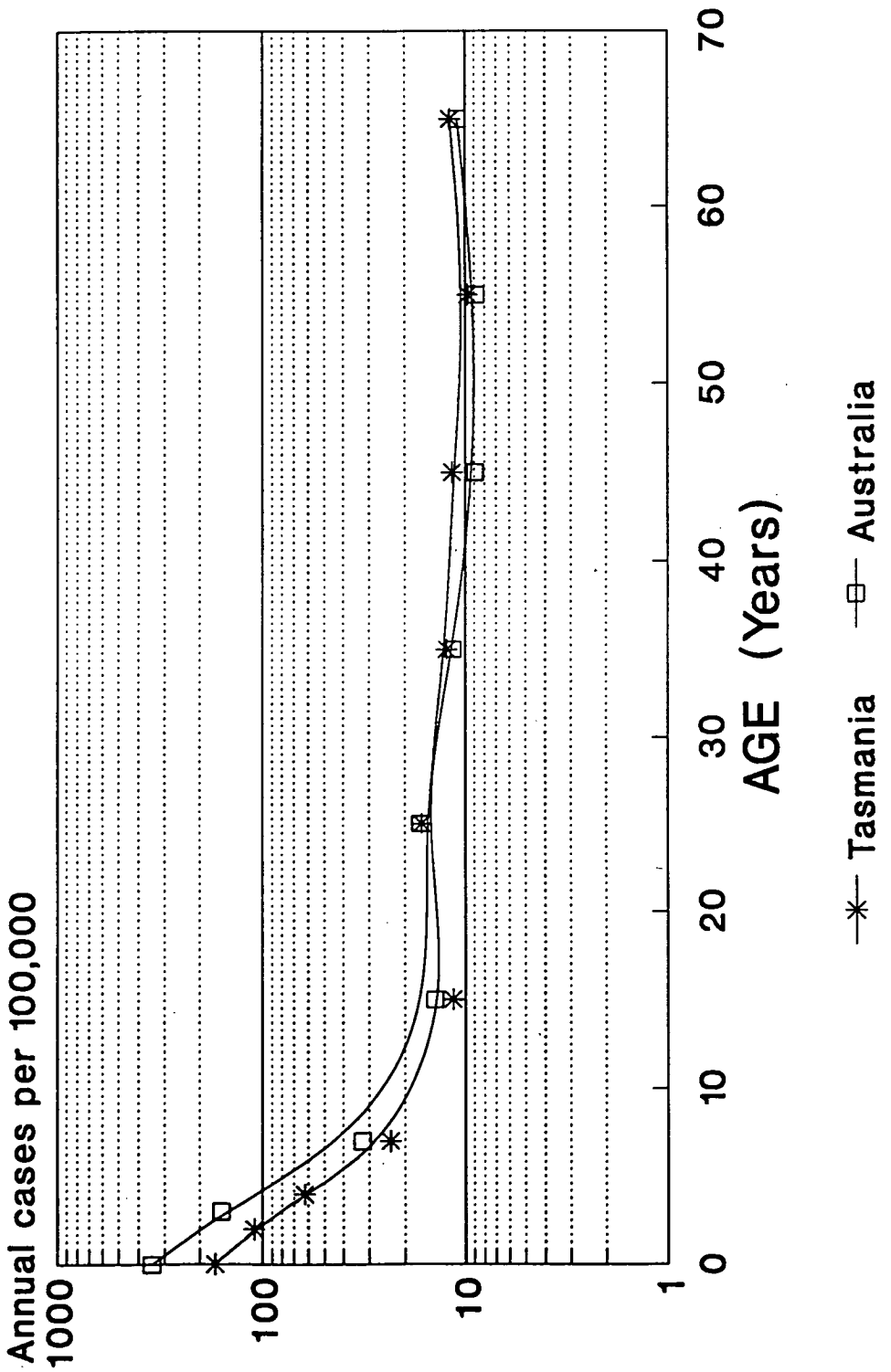
In a study into the incidence of *Salmonella* in the general population in the Hobart area faeces from 758 people displaying no gastroenteritis symptoms were tested. One case of asymptomatic *Salmonella* (*S.mississippi*) was detected, at a rate of 0.13%. This figure is very similar to asymptomatic excretion levels of 0.15% which were found in much larger studies in Japan and the UK (Cruickshank & Humphrey, 1987), but lower than those of less developed countries where levels of between 6% and 9% have been reported (Kourany & Telford, 1981; Wright, 1982).

The relative incidence of salmonellosis in various age groups within the human population generally peaks with infants, rapidly declines, and flattens out at age 5-10, after which it remains fairly constant with a slight increase in the elderly (Gorbach, 1983). The overall trends in Tasmania and Australia are similar (Fig. 10). However, there is a significantly lower incidence in infants in Tasmania compared with Australia ($P<0.005$ for infants less than 1 year old and $P<0.025$ in the 1-4 year age group).

The incidence of salmonellosis is slightly higher in males then females. In Australia 51.9% of the 25,769 cases from 1985-90 were male (NSSS). The

same proportion is observed in Tasmania where 52% of the 1120 cases occurring between 1980 and 1990 were male.

FIG. 10 HUMAN SALMONELLOSIS IN TASMANIA & AUSTRALIA: CASE RATES BY AGE



DISTRIBUTION OF *SALMONELLA* SEROVARS IN TASMANIA

Comparison of *Salmonella* serovar data from Tasmania and mainland Australia reveals several similarities and one interesting discrepancy. The frequency of *Salmonella* isolation is similar to that of most other states (Table 33). The range of serovars isolated in any single year in Tasmania is limited in comparison to that of Australia. The average number of different serovars in the 1980-90 period was 14.4 in Tasmania compared with 118 in Australia (Appendix 3 & 4). However, examination of NSSS data for this period reveals Tasmania has been accumulating serovars new to the state at twice the rate of that of Australia (7.3% per annum compared with 3.5%). The distribution of *Salmonella* serovars seen in the human populations of Tasmania and Australia are not significantly different when the relative sizes of the human populations are taken into consideration.

Salmonella typhimurium is generally the most prevalent serovar isolated from humans anywhere in the world (Van Oye, 1964). Exceptions to this do exist and are usually caused by extensive food or water-borne outbreaks. Several such outbreaks have caused one *Salmonella* serovar to dominate the annual statistics of an area, as seen recently in the UK where eggs contaminated with *S.enteritidis* were involved in a massive outbreak (Cooke, 1990). However in Tasmania, *Salmonella mississippi* has been one of the two most prevalent serovars isolated since 1979, the other being *S.typhimurium* (NSSS).

The occurrence of *Salmonella* serovars isolated in Tasmania between 1980 and 1990 is shown in Table 15. It is interesting to compare the serovars isolated from the following categories of sample: human, sewage, domestic animals and livestock, abattoirs, indigenous fauna, natural fresh water and sea water. The only two serovars which were widespread throughout the ecosystem were *Salmonella typhimurium* and *S.mississippi*, which reflects their predominance in Tasmania. In general there was good correlation between the *Salmonella* serovars isolated from wild animals, natural fresh water and sea water. Apart from *S.mississippi* and *S.typhimurium* there were no serovars common to sewage effluent and sea water whereas all marine serovars were also isolated from the indigenous fauna.

Almost half (27/61) of *Salmonella* serovars isolated from humans in Tasmania were not isolated from other sources. The serovars involved are mostly encountered infrequently in Tasmania although some of the more common Australian types occur which may be a hint as to their source. The remainder

were split into two groups which have been termed "indigenous" and "cosmopolitan" (or "exotic") by Iveson *et al* (1990), (cited by Iveson & Fleay, 1991) who noted that cosmopolitan types predominated indigenous types in the waterways of areas disturbed by human activity. This trend was also observed in Tasmania where indigenous types mostly occurred in wild animals and marine and fresh waters, and exotic types often were isolated from abattoirs and domestic/farm animals. There was very little overlap between the groups. A similar dichotomy was noted in Western Australia (Iveson & Fleay, 1991) where exotic serovars of *Salmonella* were not isolated from wild animals and only rarely from humans in less developed and sparsely populated areas.

HUMAN SALMONELLOSIS OUTBREAKS IN TASMANIA

During the course of this investigation several outbreaks of human salmonellosis have been identified and investigated. The criterion used to determine acute outbreaks was the occurrence of a particular *Salmonella* type at more than twice the normal rate, within the state, over a one or two month period.

Salmonella typhi phage type 32

During October of 1987 a patient was admitted to hospital with septicaemia. *Salmonella typhi* phage type 32 was isolated from blood and faecal culture. The patient purchased a salad roll from a local delicatessen, where the proprietor was found to be excreting the same organism. A subsequent case was also traced to the same shop. Screening of contacts revealed one of the two symptomatic patients had transmitted the disease to one of his children. The shopkeeper's family and contacts were cleared of involvement.

S.typhi phage type 32 had not before been isolated in Australia. It was postulated the carrier, who has remained in Australia since emigrating from Cyprus over 40 years ago, was a long term carrier.

Salmonella typhimurium phage type 12a

A few cases per annum of this phage type of *Salmonella typhimurium* are consistently noted in the Tasmanian population. During 1987, 46 cases were encountered throughout the state but mainly distributed along population lines (Fig.11). However, the high case rate of $170/10^5$ were reported from the

Longford - Cressy district which was significantly higher than $8/10^5$ observed in the remainder of the state. The outbreak peaked in the period between January and March, although cases were observed throughout the year. Most of the microbiological examination was carried out by the Department of Primary Industry (DPI) laboratory at Mt. Pleasant and implicated beef as the likely vehicle of infection. This organism was isolated from effluent and soil from the cattle holding pens at the Longford abattoir. Ravens feeding in this area were also discovered to be carrying *S.typhimurium* phage type 12a and *S.give*.

Salmonella typhimurium **phage type 135**

Fifteen of the 22 cases of *S.typhimurium*-135 occurring in 1989 were in March and April. This phage type is consistently isolated in Tasmania but normally not at such high levels (Appendix 3). It was found in cattle and native animals during the same period. An outbreak was investigated where several members attending a meeting became ill. Subsequently several family members of one of the victims also suffered gastroenteritis after eating left-over chicken and paté from the meeting. Testing revealed *S.typhimurium*-135 in the remaining salmon paté. The remaining paté of that batch was seized but tested negative. However, hygiene practices of the caterer were poor; chickens were thawed overnight in the sink, and there was no separation of cutlery for raw and cooked food. One of the catering staff was also found to be excreting the organism. It is difficult to assess whether chicken, some other food or the food handler was the original source. The vehicle of infection was the paté and possibly other food which had also been cross-contaminated.

The infected food handler was taking a course of antibiotics at the time of the incident and may have been predisposed to infection. She was still excreting the organism two months after being diagnosed.

Salmonella hadar

The first human cases of *S.hadar* in Tasmania were seen in the last quarter of 1990. Seven cases were identified between October and December. It was isolated from both cooked and raw chicken in one case. This serovar had also been persistent in effluent from a local chicken processing factory since January 1990. It has since been isolated in

FIG. 11 DISTRIBUTION OF HUMAN *S. TYPHIMURIUM*-12A CASES (1987)

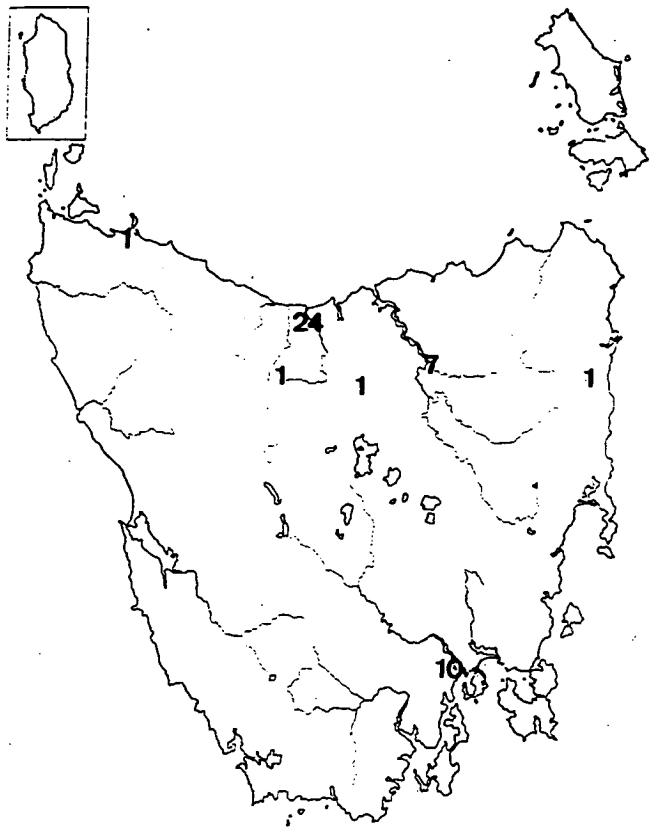
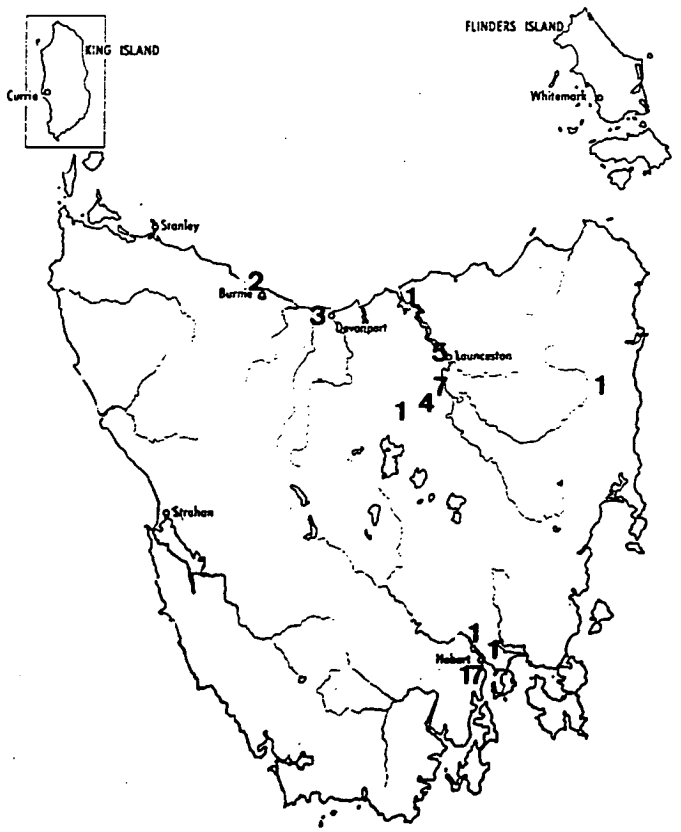


FIG. 12 LOCATION OF HUMAN *S. MISSISSIPPI* CASES (1988)



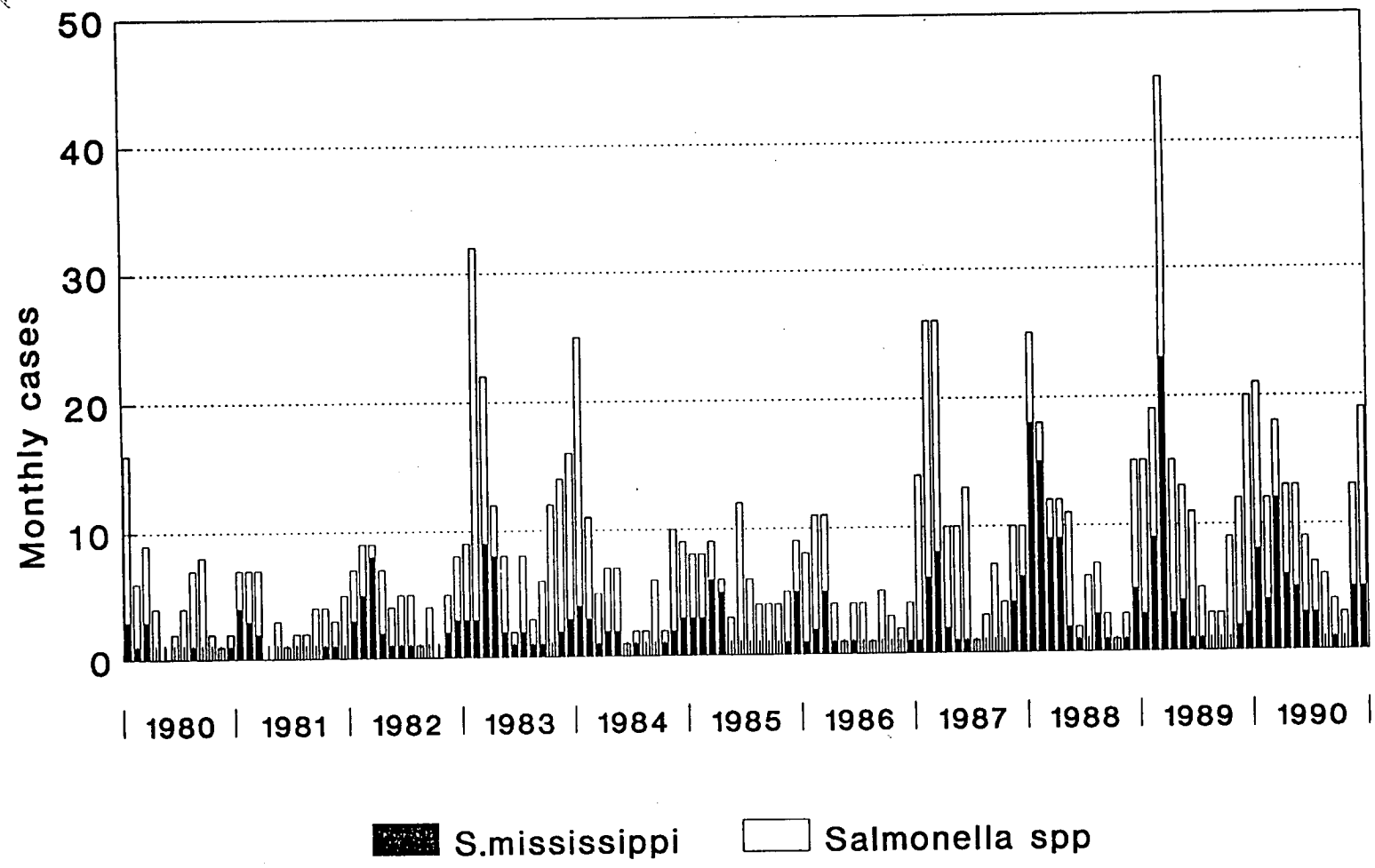
two different brands of chickens produced in both northern and southern Tasmania and processed in separate factories. Given that the same antibiotic sensitivity patterns were observed in all cases it is unlikely they originated from different sources. The lack of contact between them suggests the common source is likely to be either common breeding stock or contaminated feed. Most of the poultry feed used in commercial poultry farms in Tasmania is produced in the state and a recent survey revealed a very low incidence (1.8%) of *Salmonella* contamination in feed (Holm, 1990). However, some of the breeding stock is of mainland origin where *S.hadar* has been encountered for several years (Appendix 4).

Salmonella sofia

During 1990 *S.sofia* was first isolated in the human population in Tasmania which was followed by several notifications during the 1991 summer. This serovar was first isolated in Tasmania from chickens during 1989. It initially became apparent in Australia during 1980 in chickens and rapidly replaced *S.typhimurium* as the most frequently isolated serovar in chickens (IMVS, 1980-89) although its presence in humans was rare. *Salmonella sofia* appears to have a very low infectivity for humans while being extremely well adapted to chickens (Murray, 1991). Further evidence of poultry as the source of human infection was provided by the antibiotic sensitivities of *S.sofia* isolated from both sources which were resistant to streptomycin.

Salmonella mississippi

Increased incidence of *S.mississippi* was observed during 1988 with the peak in January-March being a considerable enhancement of the usual summer peak (Fig.13). Most cases occurred in the north of the state, focussed in Devonport with a case rate of $95/10^5$ compared with $5/10^5$ in the remainder of Tasmania (Fig.12). No common foods were implicated but most cases appeared towards the end of the drought. Many of the patients had visited areas where the reticulated water was not filtered and chlorinated. Given that some native animals frequently carry *S.mississippi* it is likely the outbreak was primarily water-borne. Both the frequency of animal carriers and the level of *Salmonella* in their excreta can be increased by drought-induced stress (Hart *et al.*, 1985). Such conditions may increase the amount of *Salmonella* in the waters of the catchment areas.



1980-1990 NSSS corrected data

FIG. 13 MONTHLY OCCURRENCE OF HUMAN *S.MISSISSIPPI* CASES IN TASMANIA (1980 - 1990)

EPIDEMIOLOGICAL INVESTIGATION OF *SALMONELLA MISSISSIPPI*

HUMAN *SALMONELLA MISSISSIPPI* CASE INVESTIGATIONS

In the initial phase of investigation as many current patients as possible were interviewed. They were questioned primarily to determine the involvement of any common foods, medical problems, water supplies or habits/practices. Contact with other people having similar symptoms and with symptomatic pets or wild animals was also noted.

Liquid manure was implicated in two cases in northern and southern Tasmania. In both cases a child became infected after eating raw vegetables from the garden. *Salmonella mississippi* was isolated from liquid manure used to fertilise the vegetable garden in each case. The liquid manure was made by mixing water with animal dung collected from pasture on which domestic and wild animals grazed and leaving it as a slurry for several weeks before use.

Drinking water was implicated in two cases involving adults in northwest Tasmania and a horse in Hobart which died of salmonellosis. In both instances creek water tested upstream of the intakes contained *S.mississippi*.

In response to an unusually high incidence of gastroenteritis reported from popular summer tourist areas, drinking water was tested on several east coast centres during peak tourist season. *Salmonella mississippi* was isolated from untreated reticulated water at Coles Bay, Port Arthur and Swansea. However, *S.mississippi* was not proven to be the aetiolic agent responsible for these incidents.

In another case of human salmonellosis, *S.mississippi* was excreted by the family dog and cat. The dog was asymptomatic but the cat developed diarrhoea a week before salmonellosis was diagnosed in the child.

A further case involved a toddler who had not been away from his suburban home during the week prior to the onset of gastroenteritis. However he was noticed eating soil and pine bark from the garden. Much later in this investigation it was discovered that skinks in the immediate vicinity carried *S.mississippi*.

To investigate the cases more fully, faecal specimens were sought from other family members. This often met with reluctance and relatively few specimens were obtained. Of the 16 families who consented to provide faecal

specimens, eight (50%) resulted in further isolations. This is higher than the 36% of households found in another study to have multiple *Salmonella* excretors (Thomas & Mogford, 1970), but probably not significantly so due to the small sample size in this investigation. Of the total of 36 contacts tested, nine (25%) yielded *Salmonella mississippi*.

Where access to patient records could be obtained or patients interviewed, a number of predisposing conditions were revealed. Prior treatment with antibiotics was noted with 7/43 patients; epilepsy in 3/40; steroid treatment in 3/40; Down's syndrome in 2/40; diabetes in 1/40; alcoholism in 1/40; and post-operative stress in 2/40. Five of the 40 hospitalised patients developed symptoms of gastroenteritis during their period of hospitalisation for unrelated reasons. In three of these cases cross-contamination was likely as they were in close proximity to patients found to be excreting *Salmonella mississippi*. This suggests that person-to-person spread may occur, especially when the recipient is compromised.

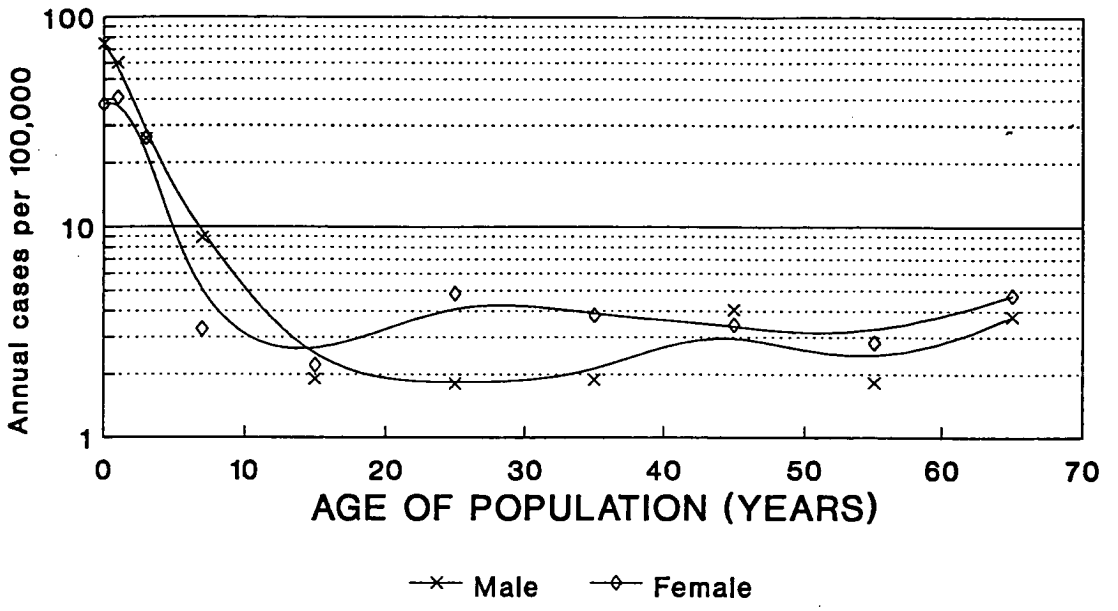
The predominant symptoms displayed by patients with *Salmonella mississippi* were those of gastroenteritis, which occurred in 86% of cases. Diarrhoea was eight times more prevalent than vomiting in such cases. A few patients presented with other symptoms; inflammatory bowel disease (1), endotoxic shock (1), post-operative pyrexia (1), and septicaemia (2). A further nine cases were asymptomatic carriers. Twenty five percent of close contacts of patients tested were found to be excreting *Salmonella*. This falls within the range of 18 to 36% of infected family members of *Salmonella* patients which has been demonstrated in other epidemiological studies (Sanders *et al.*, 1965; Thomas & Mogford, 1970; Cameron *et al.*, 1982).

The predominant type of human clinical specimen containing *S.mississippi* was faeces (324 of 334). Of the remaining specimens, vaginal swabs (3), blood (2), urine (1), sputum (1), cerebro-spinal fluid (CSF) (1), bile (1) and breast cyst fluid (1) have contained *S.mississippi*. The small proportion of non-faecal isolation sites in *S.mississippi* patients is typical of salmonellae which predominantly cause gastoenteritis in contrast to those which cause other syndromes (Wilkins & Roberts, 1988). The ratio of blood : total isolates has been used to measure the invasiveness of several *Salmonella* serovars (Wilkins & Roberts, 1988). *Salmonella choleraesuis,* with a ratio of 24-62% and *S.dublin* (40%) are regarded as most invasive and *S.panama* (6-20%) as moderately so, whereas *S.typhimurium* (0.6-5.2%) is relatively non-invasive.

Salmonella mississippi has a ratio of 0.6% which indicates it is not a particularly invasive serovar.

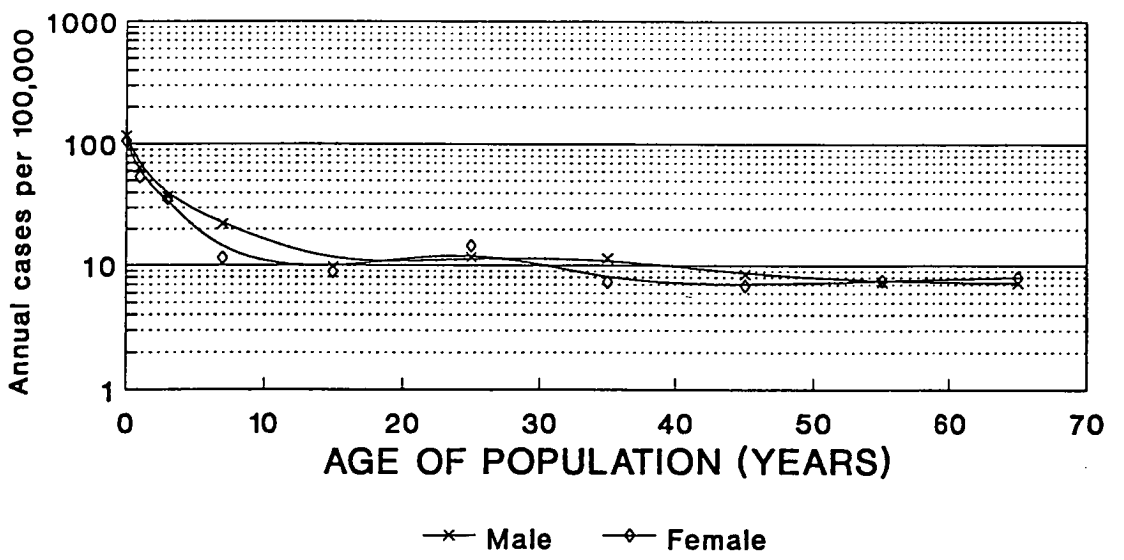
Salmonella mississippi follows the same overall pattern of age distribution as other salmonellae. Males comprised 54% of the 334 *Salmonella mississippi* cases isolated in Tasmanian during the period 1980-90 compared with 52% of all human *Salmonella* cases in both Tasmania and Australia. The distribution of *S.mississippi* between the sexes differed significantly in some age groups. *Salmonella mississippi* infection of 20-39 year old women in Tasmania was significantly higher than infection by other *Salmonella* serovars ($P<0.01$). A possible mechanism is that women of this age group are more likely to have intimate contact with infants, and infants have salmonellosis at approximately ten times the rate of the general population (Fig. 10). If person-to-person spread is implicated then this phenomenon may be explained by *S.mississippi* having a lower infective dose than that of *Salmonella* spp.

FIG. 14 CASES OF *SALMONELLA MISSISSIPPI* IN TASMANIA: AGE AND SEX VARIATION



Salmonella data from NSSS (1980-90)
Population data from 1986 census

FIG. 15 CASES OF REMAINING *SALMONELLA* SPP. IN TASMANIA: AGE AND SEX VARIATION



Salmonella data from NSSS (1980-90)
Population data from 1986 census

FIG

FIG. 16 GEOGRAPHIC DISTRIBUTION OF HUMAN *SALMONELLA MISSISSIPPI* CASES IN TASMANIA

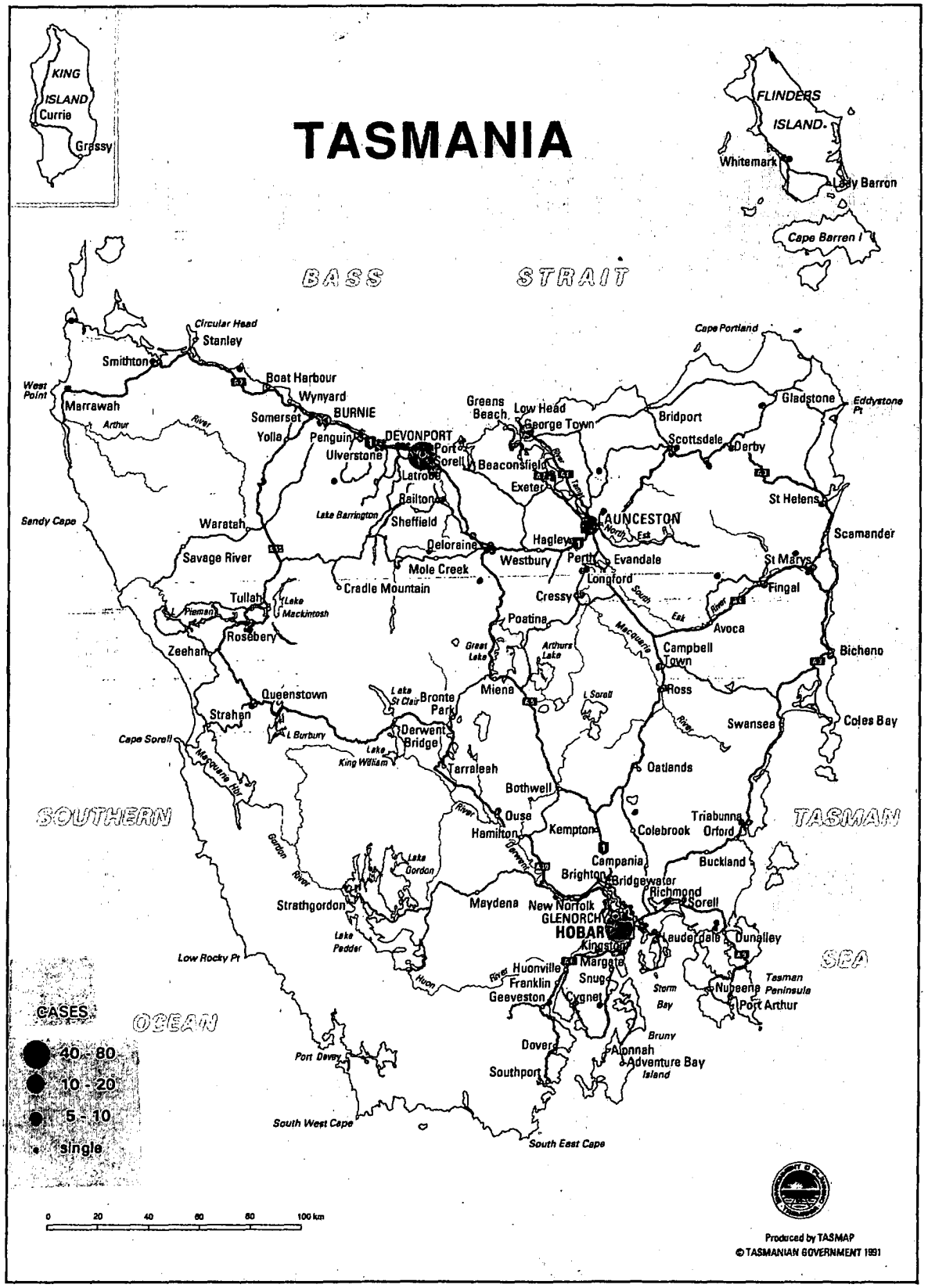
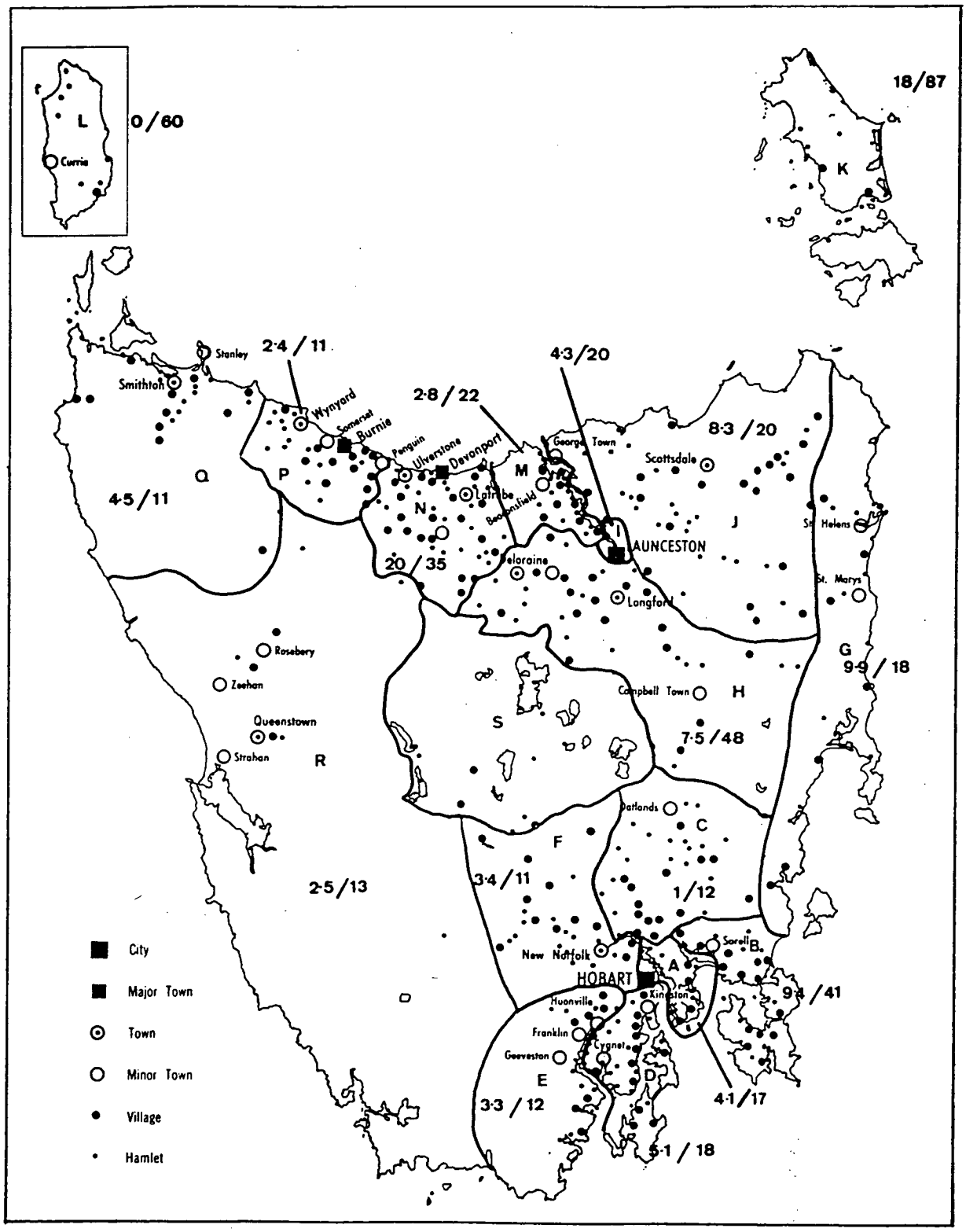


FIG. 17 PREVALENCE OF *S.MISSISSIPPI* AMONG HUMAN SALMONELLOSIS IN GEOGRAPHIC REGIONS OF TASMANIA



KEY

NUMBERS - Mean annual case rates of *S.mississippi* / *Salmonella* spp. per 10^5 population

LETTERS - Region (refer to Table 7)

FOOD SURVEYS

Data from patient interviews did not implicate any common food or food type as a vehicle of infection for *S.mississippi*. If its occurrence was perpetuated by foods it seems logical that local foods would be implicated rather than those imported to Tasmania. Several foods, which are locally produced and are predominantly consumed in Tasmania, were identified as potential sources and were surveyed.

Muttonbirds are shearwater chicks which have not yet left the burrow. Parent birds provide their food which is caught from the waters surrounding the breeding colonies in Tasmania. Muttonbirds are caught in and sold exclusively from Tasmania. The majority of fresh birds are consumed in the state and are thus a food which is uniquely Tasmanian. None of 16 fresh birds harboured *Salmonella.*

Poultry and poultry products have been implicated in many outbreaks of salmonellosis (Hobbs & Gilbert, 1978; Reilly *et al.*, 1988) due both to the high proportion of chicken carcasses which harbour the pathogen, and to inadequate cooking. Frozen chicken is more of a risk than the fresh variety if it is insufficiently thawed (Christie, 1974). Approximately half the poultry consumed in Tasmania is farmed here, the remainder being shipped (mainly frozen) from interstate. All locally grown chickens are consumed within the state (A.Demkowicz, Poultry Process Control Manager, Inghams Enterprises, Sorell. Pers. Comm.). As such they constitute a potential source of *S.mississippi*. Testing revealed 38% of locally produced chickens to be contaminated with *Salmonella*. The following serovars were isolated; *S.muenchen, S.typhimurium*-12a, *S.hadar* and *S.sofia*. *Salmonella mississippi* was not isolated from poultry.

Even though hens frequently excrete *Salmonella*, only a small proportion of eggs are contaminated (Hobbs & Gilbert, 1978). This is because eggs do not normally come into contact with faecal bacteria until after shell formation and the shells are relatively impervious to invasion. Cracked eggs lose this protective mechanism. As cracked eggs from different sources were eaten by two *S.mississippi* patients, they were surveyed but no salmonellae were isolated from the 23 samples tested.

Mushrooms are produced almost exclusively for the local market. The use of manure as a growth medium, and the warm, humid conditions, increase the hazard. However, none of the 50 samples tested grew *Salmonella*.

Sprouts of mung beans, alfalfa and similar products offer another possibility, also due to their germination in warm, moist conditions. Multiplication of *Salmonella* has been demonstrated in similar conditions (Rhodes & Kator, 1988). In some areas of production the reticulated water is of poor quality and if contaminated water were to be used in sprout production, considerable multiplication of the pathogen could occur. This is borne out by the detection of *Salmonella* and the extremely high coliform levels seen in an Australian survey of the product (National Health & Medical Research Council, 1988). No salmonellae were isolated from the 140 samples tested.

Some vegetable crops may be irrigated and are generally sprayed with river or dam water of dubious bacteriological quality. As *S.mississippi* is a common contaminant of river waters in Tasmania some crops may be the source of human infection. High proportions of salad vegetables (*ca* 70%) in an Italian survey were contaminated with *Salmonella* (Ercolani, 1976) and in a Spanish survey contaminated irrigation water was identified as the source of *Salmonella* on vegetables (Garcia-Villanova Ruiz *et al.*, 1987) although subsequent human salmonellosis was not proved. Locally grown vegetables have not been previously surveyed for *Salmonella*. Crops which are frequently eaten raw present the highest risk. Of the salad vegetables, lettuce was the highest potential problem as water may be retained in the folds of the leaves and *Salmonella* may proliferate in warm moist conditions. The risk is exacerbated if liquid compost is poured over the leaves in the belief that this will produce a healthier crop. This highly questionable practice is used by many local organic gardeners. While none of the 36 lettuces tested contained *Salmonella* there is strong epidemiological evidence that two cases were caused by eating home grown salad vegetables which had been fertilised with liquid manure.

In recent years unpasteurised milk has increased in popularity in rural Tasmania. Unpasteurised cow and goat milk were surveyed over the four years to 1991 but no salmonellae were isolated from the 43 samples tested.

Oysters grown in water polluted with sewage effluent have been implicated in human salmonellosis (Hobbs & Gilbert, 1978), although there have been no corresponding outbreaks recorded in Tasmania. It is possible a few sporadic

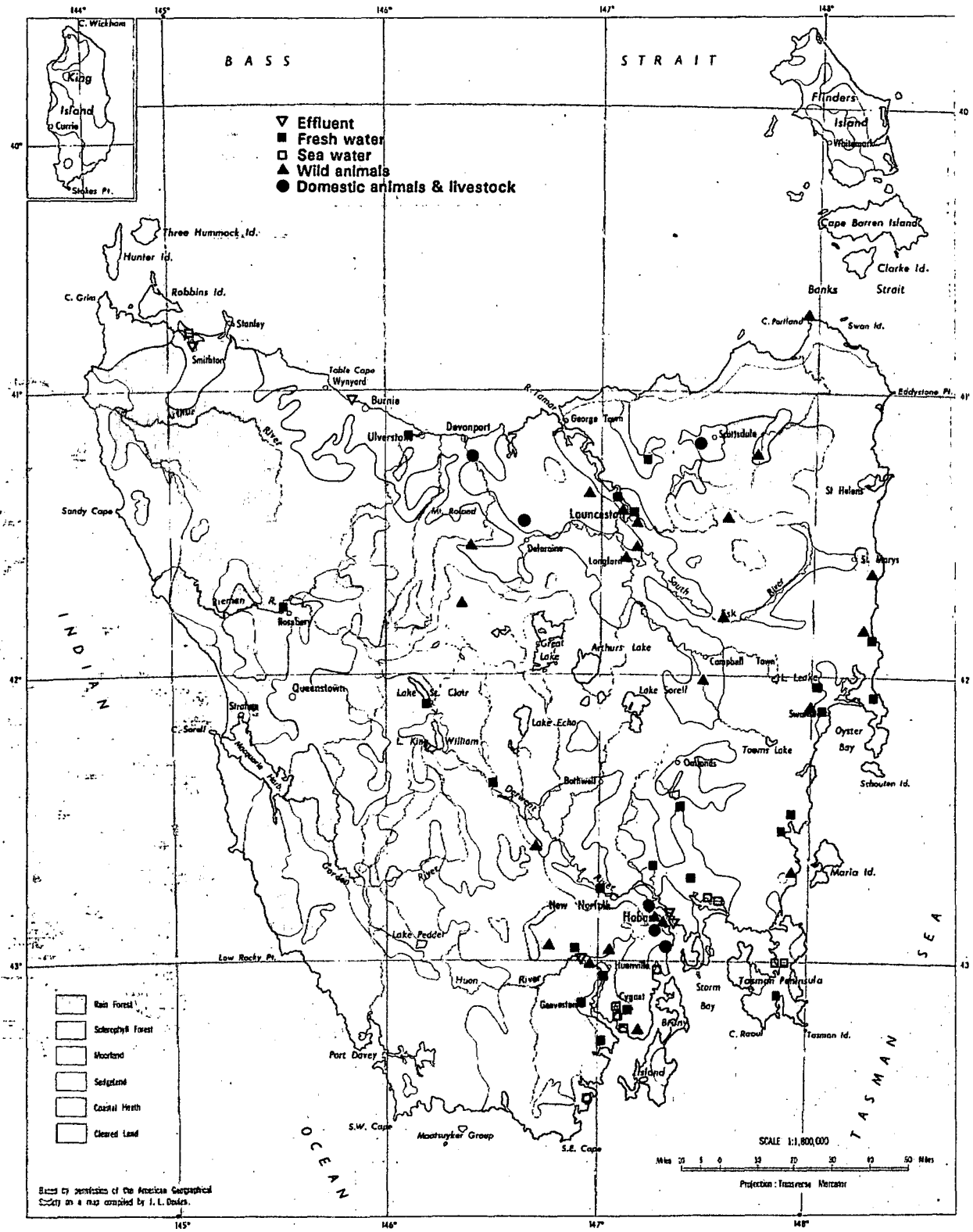
cases have been caused by the consumption of contaminated oysters. Although the quality of locally farmed oysters is well managed, one instance of *Salmonella* isolated from Tasmanian oysters marketed in Victoria was reported in 1986 (NSSS). There have been no mainland outbreaks of *S.mississippi* so it is unlikely that farmed shellfish are significant in its epidemiology. Wild shellfish, taken from locations at times when the water is heavily polluted, represent a higher potential risk but again are unlikely to constitute a major source of infection as few of the patients interviewed ate shellfish. Therefore, it is unlikely that oysters are important in the spread of *Salmonella mississippi* other than to contribute to sporadic reported cases, or others which go unreported.

Filter feeding shellfish such as oysters and mussels concentrate bacteria from the surrounding water and may be useful indicators of salmonellae in the environment and possible vehicles of human infection. In this survey *S.rowbarton*, *S.*43:d:- and *S.*(rough):43:- were isolated from locally grown oysters although *S.mississippi* and *S.adelaide* were found on previous occasions (NSSS). Of these serovars, only *S.mississippi* has been implicated in human salmonellosis in Tasmania.

Meat and meat products are also frequently contaminated with salmonellae (Johnston, 1990; Roberts, 1990). Farm animals have been surveyed by the Tasmanian Department of Primary Industry (DPI) veterinary laboratories at Launceston since 1988 and, although 17/209 (8%) cattle, 2/50 (4%) pigs and 2/110 (2%) sheep were found to contain *Salmonella*, no *S.mississippi* were isolated (DPI, unpublished data). Limited testing of some abattoir effluents during the same period revealed eight different *Salmonella* serovars: *S.senftenberg*, *S.give*, *S.derby*, *S.hadar*, *S.bovismorbificans*, *S.infantis*, *S.bredeney* and *S.mississippi*. Seven of these were isolated from the human population, reaffirming that meat was a likely contributor to human salmonellosis in Tasmania. *Salmonella mississippi* was not isolated from abattoir effluent until one instance was confirmed from the Smithton abattoir during 1991.

Feed meals of animal origin produced in Tasmania have been used in either stock pellets, interstate or overseas export, pig rations or home fertiliser (Holm, 1990). Stock feed in Tasmania is used primarily for horses and poultry; seldom for cattle or sheep. More than half the pigs produced in

FIG. 18 ISOLATION OF *S. MISSISSIPPI* FROM NON-HUMAN SOURCES IN TASMANIA



Tasmania were fed Tasmanian feed meals but as a very low incidence of porcine salmonellosis was reported in Tasmania (D.Obendorf, Veterinary Pathologist, DPI, pers. comm.), they were not considered to be a major source of salmonellosis. Nevertheless the following *Salmonella* serovars were isolated in a recent Tasmanian survey of locally produced meat, blood and bone meal (Holm, 1990); *S.derby*, *S.give*, *S.bovismorbificans*, *S.infantis* and *S.typhimurium* phage type 12, all of which were isolated from abattoir effluents suggesting a strong link between feed meals and livestock. However, *S.mississippi* was not isolated during the feed meal survey and, in view of this, feed meals were unlikely to be a significant source of *S.mississippi*.

ANIMAL SURVEYS

Animals are regarded as major reservoirs of *Salmonella*. Surveys of both domestic and wild animals were carried out in an attempt to locate possible reservoirs of *S.mississippi*

Given the assumption that *Salmonella mississippi* was primarily transmitted by food, game animals, birds and fish were surveyed. No salmonellae were found in 116 samples from the native fauna (66 macropods, 42 birds and 25 fish). The 70 samples from introduced game animals, represented by rabbits, ducks and trout, were also devoid of salmonellae.

As carnivorous animals are more likely to encounter *Salmonella* than herbivores, native carnivores were trapped and scats tested. All species tested were found to contain a large proportion of *Salmonella* carriers so their habits may be of relevance to the epidemiology.

Tasmanian devils are endemic to Tasmania and are the largest carnivores. They feed mainly on carrion and so it is not surprising that they frequently excrete a wide variety of *Salmonella* serovars. Twenty one of 36 (58%) excreted *Salmonella* of nine serovars; *Salmonella mississippi* comprised 48% of the *Salmonella* isolated.

Tiger cats remain the only true hunter of the Tasmanian native animals now that the Tasmanian tiger is thought to be extinct. They proved difficult to trap although a few of their scats were positively identified and tested. Four of five contained salmonellae, comprising two *S.mississippi* and three other serovars (Table 9).

Native cats are opportunistic carnivores with insects, especially corbie grubs, as its most important prey. They also eat ground nesting birds, small mammals, grass and fruit, and scavenge from carcasses when available (Strahan, 1983). Sixty (50%) of the 120 trapped animals were found to be excreting salmonellae. Of the 62 isolates, 60 (97%) were *S.mississippi*. The other two isolates occurred as mixed infections with *S.mississippi*. *Salmonella merseyside* was isolated from the only scat observed to contain fly maggots, which may have been its actual source. Such a specific relationship between a *Salmonella* serovar and a host animal has not been previously recorded in the literature.

A high incidence of *Salmonella* in reptiles was noted in several surveys in Australia (Iveson *et al.*, 1969; Iveson & Hart, 1983) and overseas (Matthewson, 1979; Kourany & Telford, 1981). In this survey two of four snakes and 23 of 48 lizards carried *Salmonella* of six serovars. The wide variety of serovars isolated from snakes was not surprising considering their carnivorous habits. In contrast, *S.mississippi* and *S.victoria* were the only two serovars isolated from skinks which are mainly insectivorous.

Intensive epidemiological investigation at Judbury

Salmonella mississippi was first isolated in indigenous animals from native cats at Judbury, a small farming community in a rather isolated valley in the Huon river basin. The population of approximately 100 are mostly orchardists, although some beef cattle are raised and a very small abattoir was run by one family for a few years. This area has a high density of native cats (which are timid nocturnal animals) due to its seclusion and the convoluted border between cleared pasture, where they feed at night, and sclerophyll forest where they rest during daylight hours. Once it became apparent many of the native cats were excreting *S.mississippi* the area was intensively investigated.

There was no apparent seasonal variation in carriage of *Salmonella* by native cats. Fifty two of the 78 (67%) native cats tested were *Salmonella* excretors. *Salmonella mississippi* comprised all but one of these. In an attempt to determine whether the serovar was being transmitted to native cats via infected prey some components of their diet were surveyed. No salmonellae were isolated from any of the 270 amphibians, insects or the single skink examined.

Given the abundance of native cat droppings on the pasture it was expected that some of the animals grazing in the area would be infected, but this was not the case. The incidence of scour among cattle in the area is rare and *Salmonella mississippi* was not isolated from the faeces of any of the 125 cattle, 40 rabbits, 33 wallabies or 31 pademelons tested.

Other animals at Judbury were also tested. *Salmonella mississippi* was also found in 3/7 Tasmanian devils, 1/12 possums, and 3/23 wombats. It was not isolated from 25 native hens, 5 pigs, 12 dogs, or from a solitary bandicoot or raven. However, it was isolated from two tiger snakes which were both collected within 10 km of Judbury.

Of the herbivores tested, *Salmonella* was not isolated from those which eat only grass and foliage. This contrasted with animals which had intimate contact with soil when foraging for food. It would seem that diet or feeding habits, rather than contaminated water, contributed to *Salmonella* infection of animals in this catchment area.

Salmonella mississippi was not isolated from the human population of Judbury even though it was detected in Moore swabs set in Judds creek, which serves as the source of reticulated water. It was also found in effluent from the small abattoir although not from meat, mincing equipment or swabs of the premises. However, native cats frequented the outer part of the premises so *Salmonella* could gain entry by the butcher trampling through contaminated droppings. Nevertheless, with an average slaughter of two cows per week it is unlikely to be a significant source of human salmonellosis.

WATER SURVEYS

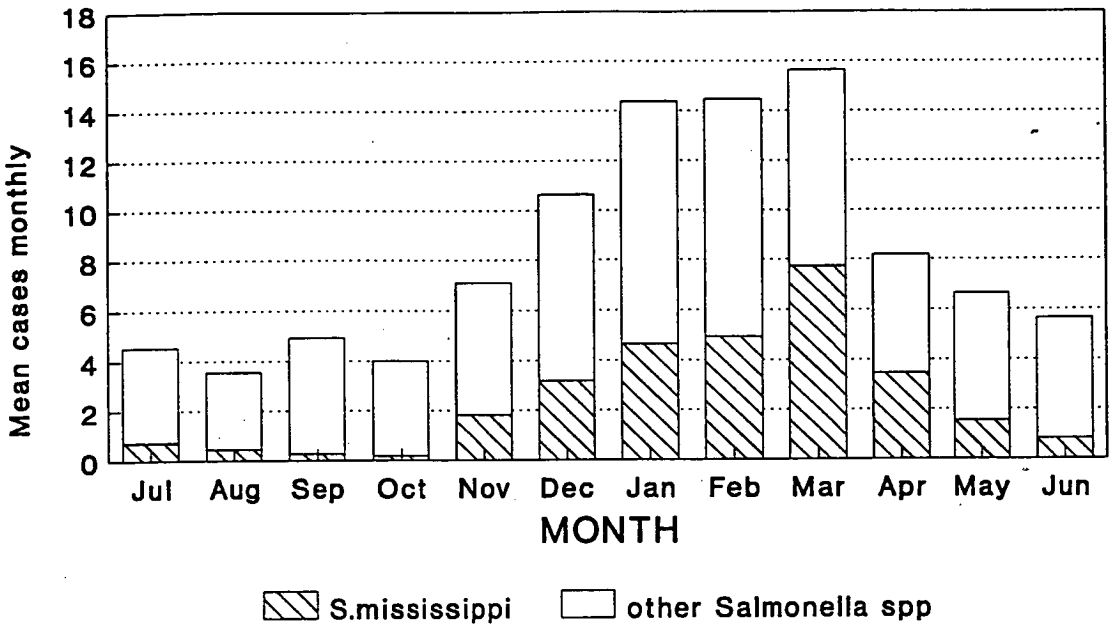
The possibility of contaminated drinking water being involved in the spread of human salmonellosis was investigated by testing water samples, received in the laboratory for routine coliform and faecal coliform enumeration, for *Salmonella* as well as indicator organisms. Tasmanian reticulated water supplies have a wide range of both treatment and quality. Supplies to cities and most of the larger towns receive both filtration and chlorination. The water quality in these areas is correspondingly high with median values of 1 coliform and <1 faecal coliform per 100mL (State Microbiology Laboratory, unpublished data). No salmonellae were detected in 100mL of any of the 428

potable water samples tested in the period 1984-1991. However, not all reticulated water is of such high quality. In about three quarters of towns in the 500-10,000 population range, water is not adequately treated. Some supplies receive filtration only, others chlorination without prior filtration, and others reticulate untreated water. For populations below 500 very few supplies receive any treatment. Overall, 9 of 556 (1.6%) reticulated water samples were found to contain *Salmonella*. Furthermore, natural river and dam waters were surveyed resulting in an isolation rate of 7.2% from the 319 samples tested. The situation is comparable to West Australian where 1.6% and 10% of drinking water in metropolitan and country areas respectively were contaminated with *Salmonella* (Peterson & Schorsch, 1980).

The presence of salmonellae in natural water bodies was not unexpected. Their source was presumably faecal, either of human or animal origin. Thus the serovars isolated (Table 12) were a reflection of those present in animals and man in the catchment area. *Salmonella mississippi* comprised 28/47 (60%) of these, further confirming the significance of this serovar in Tasmania. In several instances salmonellae were isolated in areas devoid of human habitation and domestic animals and can be attributed directly to the native fauna in the catchment. The serovars isolated in such circumstances included *S.mississippi, S.victoria, S.houten, S.warragul* and several *Arizona* spp. Similarly, wild animals were reported to be the source of indigenous salmonellae encountered in water supplies of rural West Australia (Peterson & Schorsch, 1980).

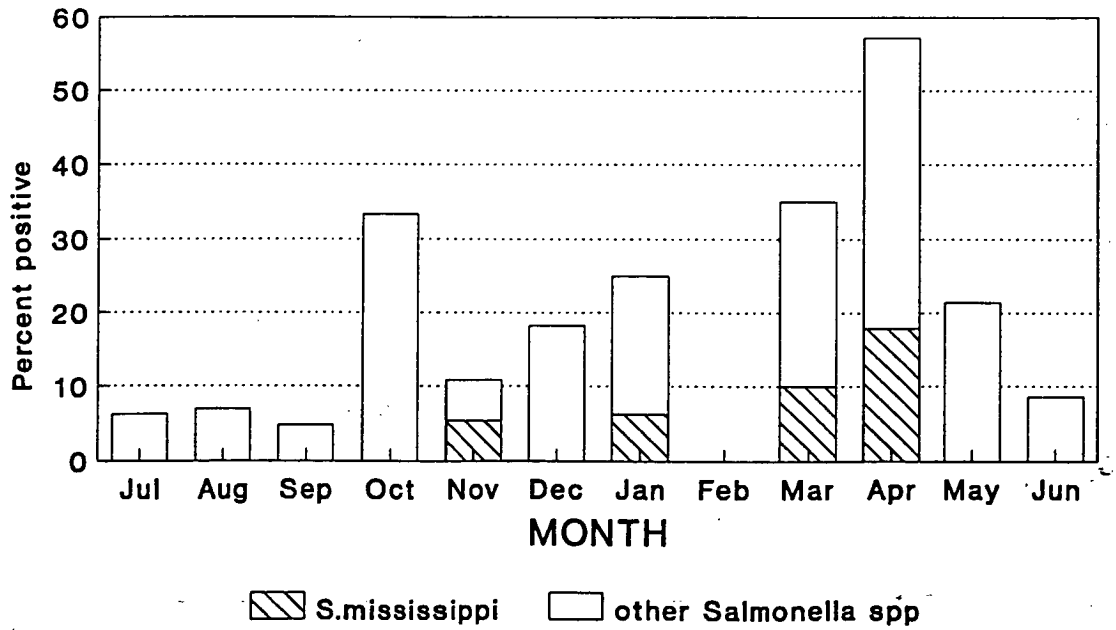
From observations of the seasonal distribution of *Salmonella* in fresh water, sea water and sewage an interesting pattern emerged. In natural fresh waters *Salmonella mississippi* first became apparent during September and was at a peak in November - December and remained prominent until March (Fig.19). The occurrence of *S.mississippi* in Tasmanian waterways preceded its occurrence in sea water, sewage and the human population. There was a lag period of a month before it was seen to the same extent in sea water (Fig.20), a further month for human manifestation (Fig.21) and a further month for sewage (Fig.22). The lag period from river to sea, and human to sewage was expected but progression to humans supports the hypothesis of contaminated water being an important vehicle of *Salmonella mississippi* infection, especially since the same trend did not exist for other *Salmonella* spp.

FIG. 21 MONTHLY VARIATION IN HUMAN SALMONELLOSIS



Corrected NSSS data (1980-90)

FIG. 22 MONTHLY OCCURRENCE OF *SALMONELLA* IN SEWAGE



GROWTH & SURVIVAL OF *SALMONELLA MISSISSIPPI*

As well as their source in animals, certain *Salmonella* serovars may become prominent if they are able to grow or survive in the environment better than others. To this end the survival of several strains of *Salmonella* was monitored under certain conditions.

SURVIVAL

Salmonella mississippi persisted longer in fresh water than in sea water and had decimal reduction times (D values) in water and sea water comparable to those of other salmonellae. At 15°C the range of D values for *S.mississippi* in sea water was 2.3 to 6.0 days (mean of 3.8), compared with 4.5 days for *S.typhimurium* (Vasconcelos & Schwartz (1976) cited by Mitscherlich & Marth, 1984). Decimal reduction times of *S.mississippi* in fresh water were between 1.1 and 1.8 days at 30°C compared with the 0.33 - 2.2 days for various salmonellae found by others (Mitchell & Starzyk (1975) cited by Mitscherlich & Marth, 1984). The authors also reported D values of 16 days and 7-8 days at 5°C and 10-20°C respectively, which were similar to the ranges observed for *S.mississippi* in this study of 6.7-21 days and 3.0-5.8 days at comparable temperatures. It should be noted that all of these experiments enumerated *Salmonella* by conventional methods. They did not consider the possibility that *Salmonella* may change to VNC forms and thus remain potentially infectious (Singh *et al* (1986) cited by Desmonts *et al.*, 1990), rather than dying. The practical relevance of D values in these circumstances is therefore questionable.

There is a wealth of information on the survival of bacteria in various environments (Mitscherlich & Marth, 1984). For an element of the environment to be a factor in the selective survival of *S.mississippi* it must differ between Tasmania and mainland Australia. Two such factors are temperature and sunlight. Tasmania has lower optimum and minimum temperatures than most of mainland Australia. The depletion of the ozone layer is more pronounced over Tasmania than areas further north so the amount of UV is higher in Tasmania than elsewhere in Australia.

The effect of sunlight on the survival of washed cell suspensions of various strains of *Salmonella* in sterile water was measured after a full day of exposure to the sun. The preliminary results of this experiment displayed wide variability. However, a significant difference was observed between the

survival of *Salmonella mississippi* and *S.choleraesuis*, an invasive serovar. The mean percentage survival rate of nine strains of *S.mississippi* was 18% (SD = 10%) compared with 12% (SD = 13%) of 15 other salmonellae although the mean values were not significantly different. However, the strain variation seen in the response of *S.mississippi* to sunlight was smaller than that displayed by mixed serovars which suggests that there may be more variation between serovars than strains of a serovar. The differing abilities of various *Salmonella* serovars to tolerate solar radiation may contribute to the presence of "environmental strains" of *Salmonella* alluded to by some workers (Murray, 1991). Further trials need to be undertaken to determine the significance of this effect and if it contributes to the prevalence of *S.mississippi* in the environment.

Soil is another component of the Tasmanian environment which may differ from mainland Australia. The major environmental stresses to which bacteria are exposed in soil are physico-chemical and biological in nature (Mitscherlich & Marth, 1984). To the former belong lack of available water, unfavourable oxygen tension, pH and temperature; to the latter belong poor nutrient availability and the autochthonous soil microbiota. It is impossible to draw any conclusions on relative survival rates of the four *Salmonella* serovars due to the highly irregular pattern of recovery from the three soils over time. This may well be due to the non-homogeneous distribution of different microcosms within plots where various of the factors mentioned above may differ. This is a problem inherent *in vivo*, due to the complex interplay of factors involved, and makes accurate comparisons of the survival of salmonellae in soil very difficult. Nevertheless, *S.mississippi* was readily recovered from clay loam for one month and sporadically for a further month and so contaminated soil may be a source of infection for two months after being contaminated.

GROWTH

The minimum and optimum growth temperatures for *Salmonella mississippi* were observed to be 7.7°C and 39°C respectively (Fig.5). These cardinal growth temperatures fell within the expected range of *Salmonella* (Petzold & Scheibner (1965) cited by Mitscherlich & Marth, 1984). However, the theoretical minimum growth temperature (T_{min}) was calculated at 3.2-3.4°C, which was also comparable to T_{min} values of 3.24-3.78°C observed by several *Salmonella* serovars grown on minced beef (Smith, 1985; Mackey & Kerridge, 1988). Some ecological advantage may arise in Tasmania's

relatively cold climate were *S.mississippi* to grow at lower temperatures than competitive serovars. To investigate this possibility the minimum growth temperatures of eight strains of *S.mississippi* and ten other *Salmonella* serovars were determined within 0.5°C. All 18 cultures grew at 7.0°C while none grew within three weeks at 5.5°C. The minimum growth temperature of six of the eight *S.mississippi* strains fell between 6.0 and 6.5°C, whereas one grew at 6.0°C and the other did not grow at 6.5°C. In comparison three of the ten other serovars tested were capable of growth at 6.5°C although 30 of the 40 *Salmonella* serovars tested by Petzold & Scheibner (1965), (cited by Mitscherlich & Marth, 1984) grew at this temperature. *Salmonella mississippi* was not significantly different to other salmonellae with respect to minimum growth temperature.

THE ECOLOGY OF *SALMONELLA MISSISSIPPI* IN TASMANIA

It is unclear when *Salmonella mississippi* became established in Tasmania because *Salmonella* serotyping has only been undertaken in Australia since 1937 (Fenner, 1990) and in Tasmania *Salmonella* serology was rarely carried out before 1970 (Dr.L.Lyons, Department of Health, pers. comm.). The first isolation of *S.mississippi* occurred in Florida during 1943 (Edwards *et al.*, 1943) and it first became apparent in Australia one year later in northern Australia. It is possible it was introduced from one of these locations to the other and subsequently became established in the respective resident wild animal populations. Such a scenario would require very rapid dispersal to become widespread so quickly in both Tasmania and southern USA. It is more likely the serovar was not recognised before this due to the stage of development of *Salmonella* serotyping at the time.

It is interesting to note that in the three global geographic locations where regular isolations of *S.mississippi* occur; southern USA, New Zealand and Tasmania, marsupials are a component of the local fauna. Marsupials dominate the mammalian species in Australia, but are sparsely represented in the other two locations. Opossums of the USA are not found in Oceania but the possums in New Zealand were introduced from Australia. Another possible link between *S.mississippi* and fauna are scincid lizards. Skinks of the genus *Leiolopisma,* some of which were recently reclassified as *Niveoscincus* (Hutchinson *et al.*, 1990), are common to Tasmania, New

Zealand and America (Smith, 1946). The common skink (*Leiolopisma zelandica*) was identified as the reservoir of *Salmonella saintpaul* which was seen in a disproportionate number of human salmonellosis cases in the Otago district of New Zealand (de Hamel & McInnes, 1971). On the American continent they are distributed over eastern USA and extend south to Panama, which corresponds to the distribution of *S.mississippi* in the USA (CDC, 1990). They are active in Oklahoma from April to October (Smith, 1946) which precedes the peak incidence of human *S.mississippi* salmonellosis by one month (CDC, 1990). The same situation occurs in Tasmania.

Salmonella mississippi is present in the native cat populations of northern and southern Tasmania. Between 20% and 64% of the native cat population was infected with this organism to the virtual exclusion of other salmonellae, which is not simply caused by the predominance of *S.mississippi* in the ecosystem. If this were the case *S.mississippi* would similarly dominate *Salmonella* surveys of other animals which it clearly does not. Tasmanian devils, which are similar to native cats in both distribution and many dietary components, displayed 11 *Salmonella* serovars. There are two possible explanations for the phenomenon of predilection of native cats to one *Salmonella* serovar; a bacterial/host affinity or a contaminated dietary component.

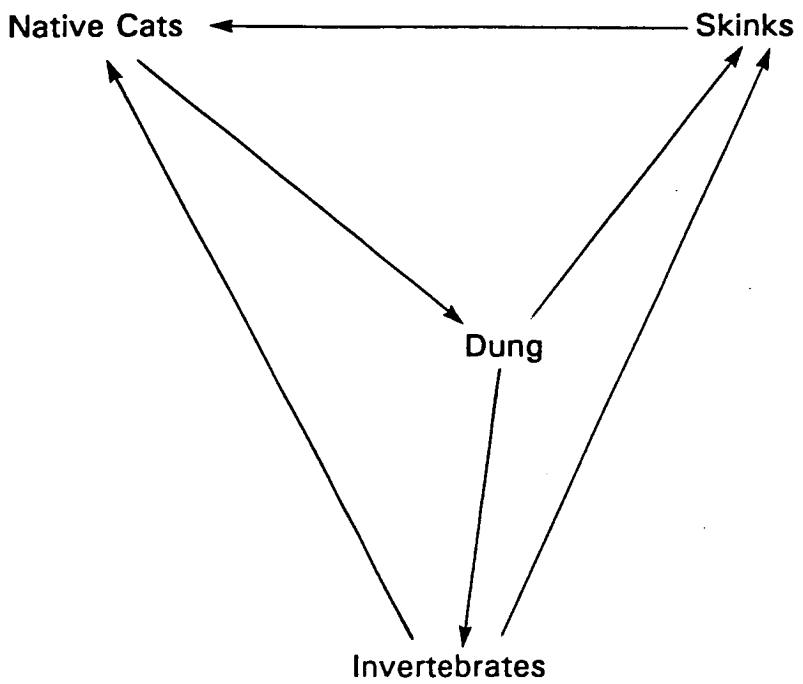
An affinity between *S.mississippi* and native cats would explain the prolonged excretion period observed in feeding trials. *Salmonella mississippi* persisted for at least 11-16 weeks compared with excretion periods of 1-6 weeks after native cats were inoculated orally. The same effect was not noted with brushtail possums which displayed no such serovar specificity. As skinks were only recently identified as a major source of *Salmonella mississippi* their long term carrier status is yet to be determined. *Salmonella mississippi* does not appear to harm its host which may benefit from the association if infection by other potentially more pathogenic *Salmonella* spp. can be prevented. However, stress in animals has been shown to increase the proportion of animals carrying *Salmonella*. This phenomenon was described for sheep stressed by starvation or long periods of transportation (Moule & Young, 1951), quokkas during drought-induced starvation (Iveson & Hart, 1983), cattle and horses after prolonged transportation (Atkinson, 1964), horses after surgery (Hird *et al.*, 1986) and poultry in overcrowded conditions (Atkinson, 1964). Although stresses due to drought, overcrowding, competition and starvation were eliminated, it is still possible the prolonged period of *Salmonella* shedding was an artifact of stress caused by captivity. However,

all captive animals became progressively more manageable which is a sign of reduced stress.

Alternatively native cats may be continually reinfected during feeding. If so there must be either another animal which is the main permanent reservoir of *S.mississippi* or it is circulated between native cats, a small number of other hosts and the local environment. One such animal is the metallic skink which had a 62% infection rate with *S.mississippi* which comprised 95% of the *Salmonella* isolated from them. The skink may be the natural reservoir of *S.mississippi* or the infection may be perpetuated by a local effect such as diet. The proportion of native cats and metallic skinks excreting *S.mississippi* varied with locality. At Judbury, 64% of captured native cats excreted *S.mississippi* compared with 20% of those from other locations. A similar situation was observed in several provinces in New Zealand with skinks carrying *S.saintpaul* (de Hamel & McInnes,1971). Involvement of an infected dietary component which is irregularly distributed may explain this phenomenon. As both skinks and native cats are insectivorous a preliminary survey of insects was undertaken but failed to isolate *Salmonella*.

Native cats and skinks may be part of a small cycle involving *S.mississippi*. *Salmonella* can persist for several months in faeces (Josland, 1951). There is an abundant supply of native cat scats in areas such as Judbury where the animal is common. During the warmer months faecal pellets are rapidly dispersed by insects and worms etc. Skinks are active during the same period and may feed on some of the invertebrates involved in dung dispersal. Native cats prey on skinks and insects, thereby completing the cycle. However, this does not explain the predominance of *S.mississippi* or the exclusion of other serovars of *Salmonella* from the local ecosystem.

FIG. 23 POSSIBLE CYCLE OF *S.MISSISSIPPI* AMONG NATIVE FAUNA



The role of insects as vectors of disease was recognised as long ago as 1903 (Hamilton (1903) cited by Steinhaus, 1947). Small proportions of flies, ants and cockroaches surveyed (Singh *et al.*, 1980) were found to carry *Salmonella*. Insects are generally regarded as transient carriers (Steinhaus, 1947, Pierce (1921), Thomson (1913) cited by Mitscherlich & Marth, 1984). However the possibility of insects being long term carriers of *Salmonella* has received little consideration although it was known to be excreted for 42 days by infected cockroaches (Mackerras & Pope, 1948) and 15 days by flies (Gross & Preuss (1950) cited by Mitscherlich & Marth, 1984) and beetles (Geissler & Kosters (1972) cited by Mitscherlich & Marth, 1984). *Salmonella eimsbuettel* was shown to persist in a closed system containing bread beetles (*Stegobium panaceum*) and sterile fodder for five years (Cuturnik & Topolnik (1975) cited by Mitscherlich & Marth, 1984). The possibility of a long term association between an insect and a *Salmonella* serovar arose from an investigation into human salmonellosis caused by carmine dye infected with *S.cubana* (Lang *et al.*, 1967). As carmine is a pigment produced by cochineal beetles (*Dactylopius coccus costa*) they were tested and found to be carrying

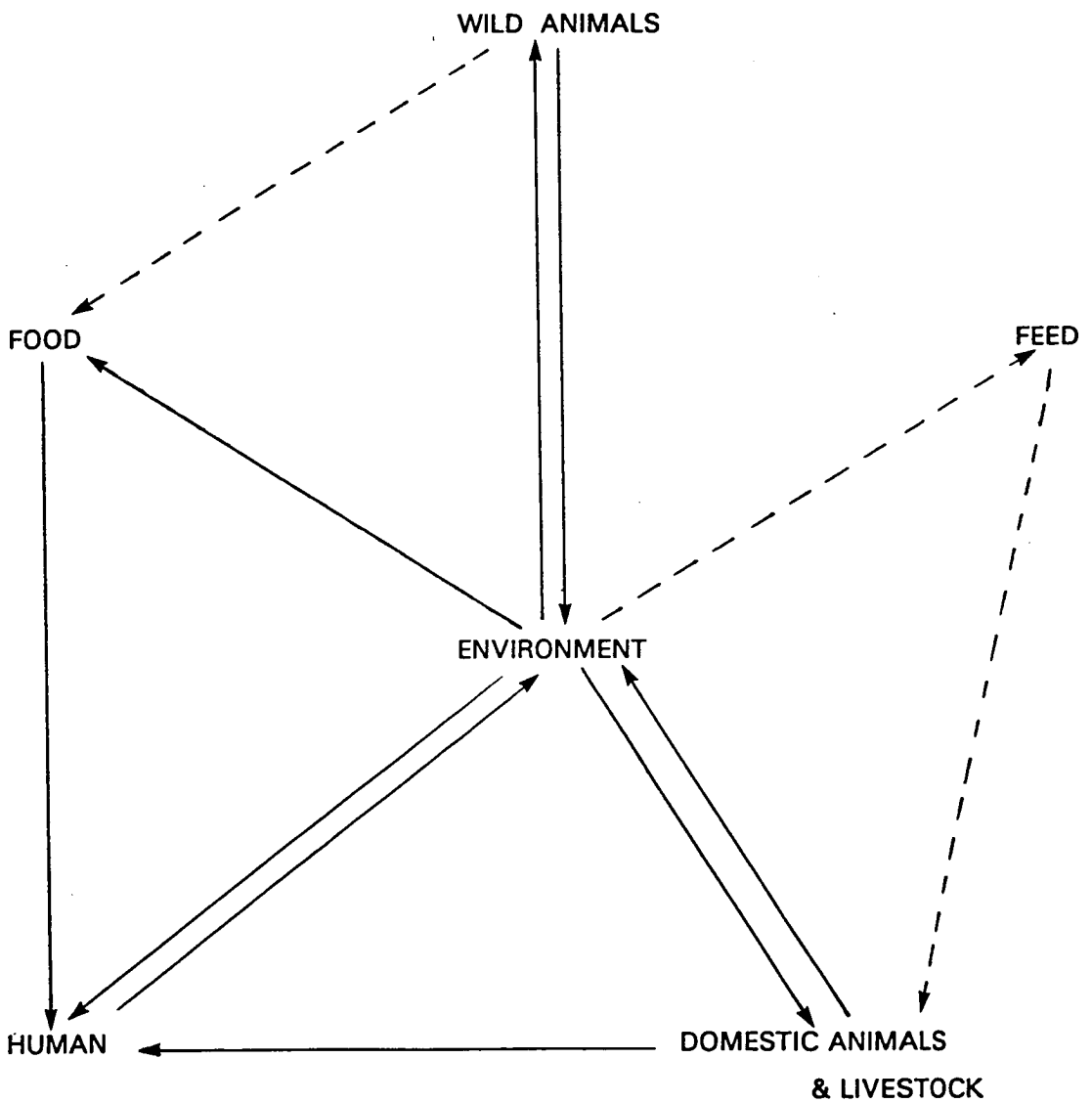
the pathogen. Cochineal beetles from both Peru and the Canary Islands, the major areas of carmine production, were colonised with *S.cubana*. The beetle and the prickly pear cactus (*Nopalea cochinellifera*) on which they primarily feed were introduced to both locations from central America over 150 years ago. The colonies have been geographically separated since. This suggests an affinity between the insect and the *Salmonella* serovar. It is interesting to note both *S.cubana* and *S.mississippi* are group G2 salmonellae and share the somatic antigenic structure, 1,13,23. Members of this group are not commonly encountered and were isolated from only 2014/60659 (3.3%) of salmonellosis cases in Australia between 1980 and 1990 (NSSS). It is possible they may share adhesins which selectively bind to some insect antigen. This may warrant further investigation if insects are implicated as carriers of *S.mississippi* in future studies.

SALMONELLA MISSISSIPPI CYCLE

It is doubtful whether native cats are involved in the direct transmission of *Salmonella mississippi* to humans as they are timid creatures and have scant contact with humans. Infection from this source is most likely to be via contaminated drinking water. Conversely, metallic skinks are widespread in urban areas throughout Tasmania and hence have close human contact. Disease transmission may occur as a result of direct contact by patients handling the skinks as very high levels of *S.mississippi* (10^9/g) have been observed in fresh skink faeces. Food poisoning may occur as a result of faecal contamination of vegetables as skinks forage for insects and other prey readily found in gardens. A further possible infection mechanism is by cats which commonly catch skinks and may thus become infected and take the *Salmonella* indoors to infect their owners. This avenue is being investigated further.

Water contaminated with *Salmonella mississippi* is possibly the most likely source of infection in the many towns in Tasmania which are supplied with inadequately treated reticulated water. Such a mode of transmission has been demonstrated on two occasions and is supported by the peak human occurrence being immediately preceded by elevated levels of *S.mississippi* in water. Low numbers of *Salmonella* in contaminated drinking water may be sufficient to cause

FIG. 24 *SALMONELLA MISSISSIPPI* CYCLING IN TASMANIA



infection as ingestion of small amounts (<50mL) of water may have diminished contact with stomach acid (Mossel & Oei, 1975).

Person-to-person transmission was demonstrated in two instances involving mothers and new-born infants. Tasmanian women in the 20-39 age group are infected with *Salmonella mississippi* at a significantly higher rate than by other *Salmonella* spp. or by men of the same age group. It is possible that this disparity may be due to increased contact of mothers with infected children and a lower infective dose.

In a further two cases infection was food-borne after home grown vegetables were fertilised with contaminated liquid manure.

WATER-BORNE *SALMONELLA* IN TASMANIA

Routine testing of drinking water for pathogens is both expensive and time consuming and has not been done in Tasmania. To assess the disease risk to the human population drinking water is usually tested for the presence of the indicator organisms: coliforms, faecal coliforms and faecal streptococci. The presence of one faecal coliform per 100mL is the maximum permissible limit applied to potable waters (NH&MRC & AWRC, 1987). The level of faecal coliforms in reticulated and natural water gave a good indication of the probability of the water containing *Salmonella* (Fig.25). However, the absence of faecal indicator bacteria did not entirely preclude the presence of *Salmonella*, as indicated by McNeil (McNeill, 1985). *Salmonella* was isolated from two such waters in the course of this investigation.

Examination of Figures 26 - 28 revealed *Salmonella* to be more abundant in natural and marine waters than in wastewater of equivalent bacterial indicator quality. This phenomenon has been noted previously (Kott (1977) cited by McNeill, 1985) and may have two possible causes. The first was a difference in the ratio of *Salmonella* to indicator bacteria between human and animal excreta. The second was a difference in growth or survival patterns in the environment. The *E.coli* component of coliforms decreased from over 90% in human and animal faeces to approximately 20% and 30% in raw and treated sewage effluent respectively (Dufour (1977) cited by McNeill, 1985). Differential survival was shown in preliminary survival experiments where the survival rate of *S.mississippi* was approximately ten times that of *S.choleraesuis* and *E.coli* in fresh water exposed to sunlight. A similar effect

was seen in estuarine water (McCambridge & McMeekin, 1981). The decay of *S.typhimurium* and *E.coli* were not significantly different in sea water and fresh water (Davies & Evison, 1991). Preliminary experiments have shown that the effect of sunlight on the survival of some *Salmonella* serovars approximated that of *E.coli*, but other *Salmonella* serovars were more tolerant to sunlight (Tables 25-29). Further work in this area is necessary to determine whether this effect is a property of particular serovars of *Salmonella* or whether wide strain variation exists within *a Salmonella* serovar, and whether *E.coli* also displays wide strain variation.

Salmonella excreted from infected animals can remain viable for several months in faeces (Drescher & Hopfengarter (1933) cited by Mitscherlich & Marth, 1984) and soil (Thunegard (1975) cited by Mitscherlich & Marth, 1984) from which it may enter water courses from runoff after rain. *Salmonella* can remain viable in water for days to weeks (McFeters *et al.*, 1974, Drescher & Hopfengarter (1933) cited by Mitscherlich & Marth, 1984) and possibly longer in VNC form. In this way *Salmonella* of animal origin (mainly *S.mississippi* in Tasmania) may cause sporadic human infection. The most likely period for water-borne human salmonellosis to occur would be after heavy rain, especially after a prolonged dry spell (Haddock & Malilay, 1986; Wright, 1986). Animal dung and associated pathogens, accumulated during the dry period, are flushed into the waterways in these circumstances. At such times reticulation systems lacking filters are likely to fail as the increased particulate and organic load render chlorination ineffective.

The low infective doses from ingestion of small amounts of contaminated water, the poor bacteriological quality of many Tasmanian reticulated water supplies, and the likely presence of undetected VNC forms all contribute to the likelihood of water as a significant cause of salmonellosis in Tasmania.

FIG. 25 RELATIONSHIP BETWEEN *SALMONELLA* AND INDICATOR BACTERIA IN FRESH WATER

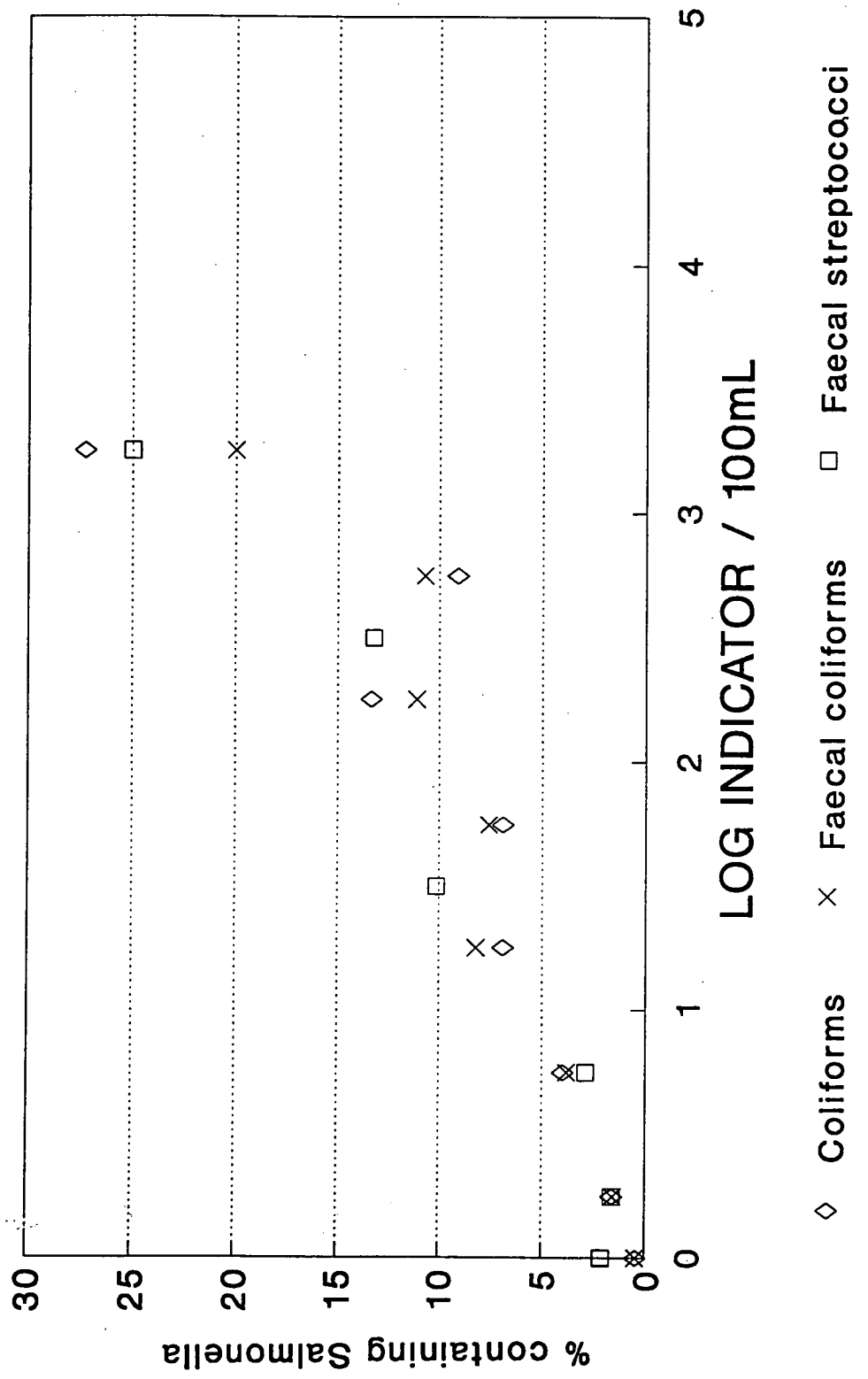


FIG. 26 RELATIONSHIP BETWEEN *SALMONELLA* AND COLIFORMS IN FRESH WATER, SEA WATER AND WASTEWATER

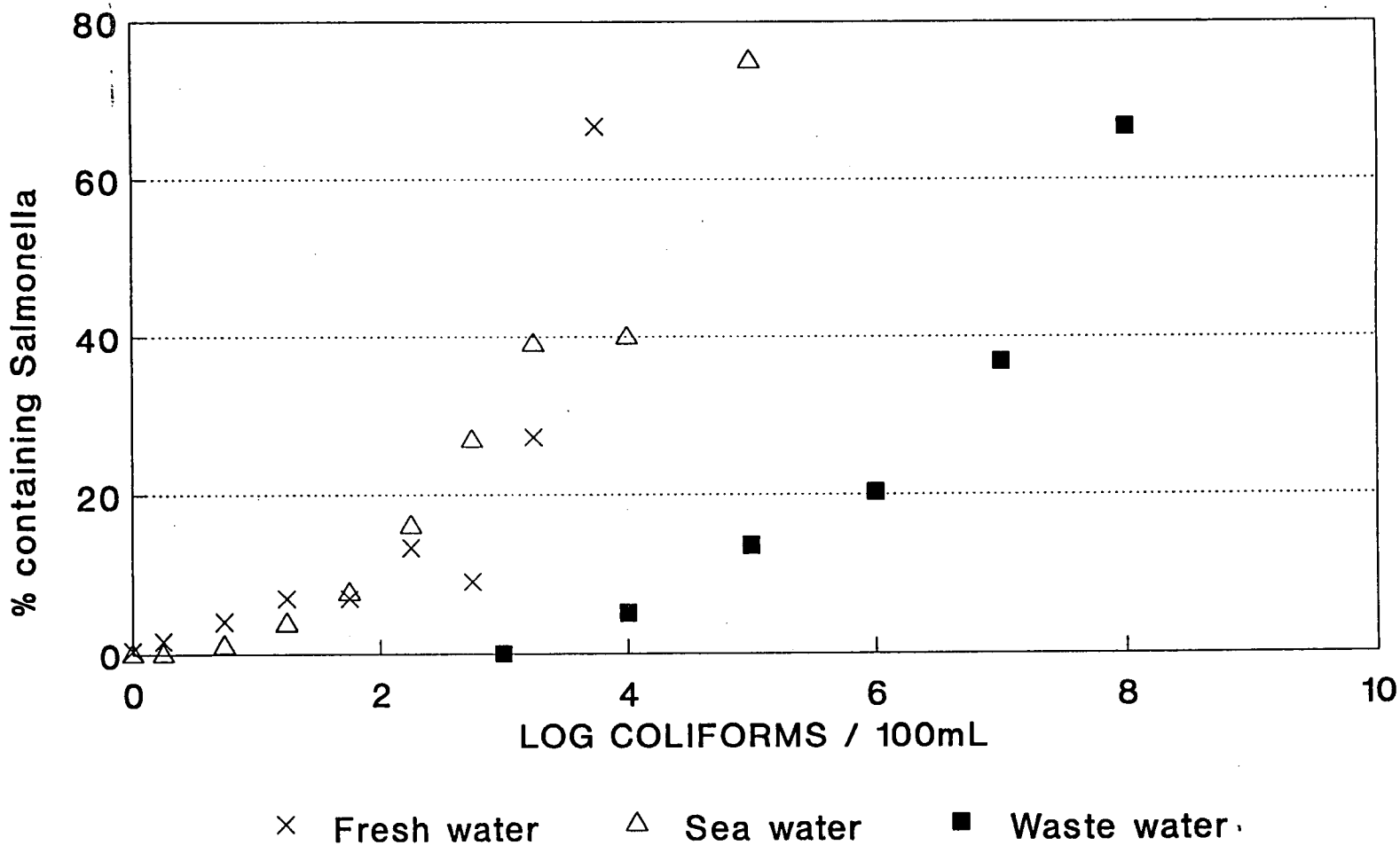


FIG. 27 RELATIONSHIP BETWEEN *SALMONELLA* AND FAECAL COLIFORMS IN FRESH WATER, SEA WATER AND WASTEWATER

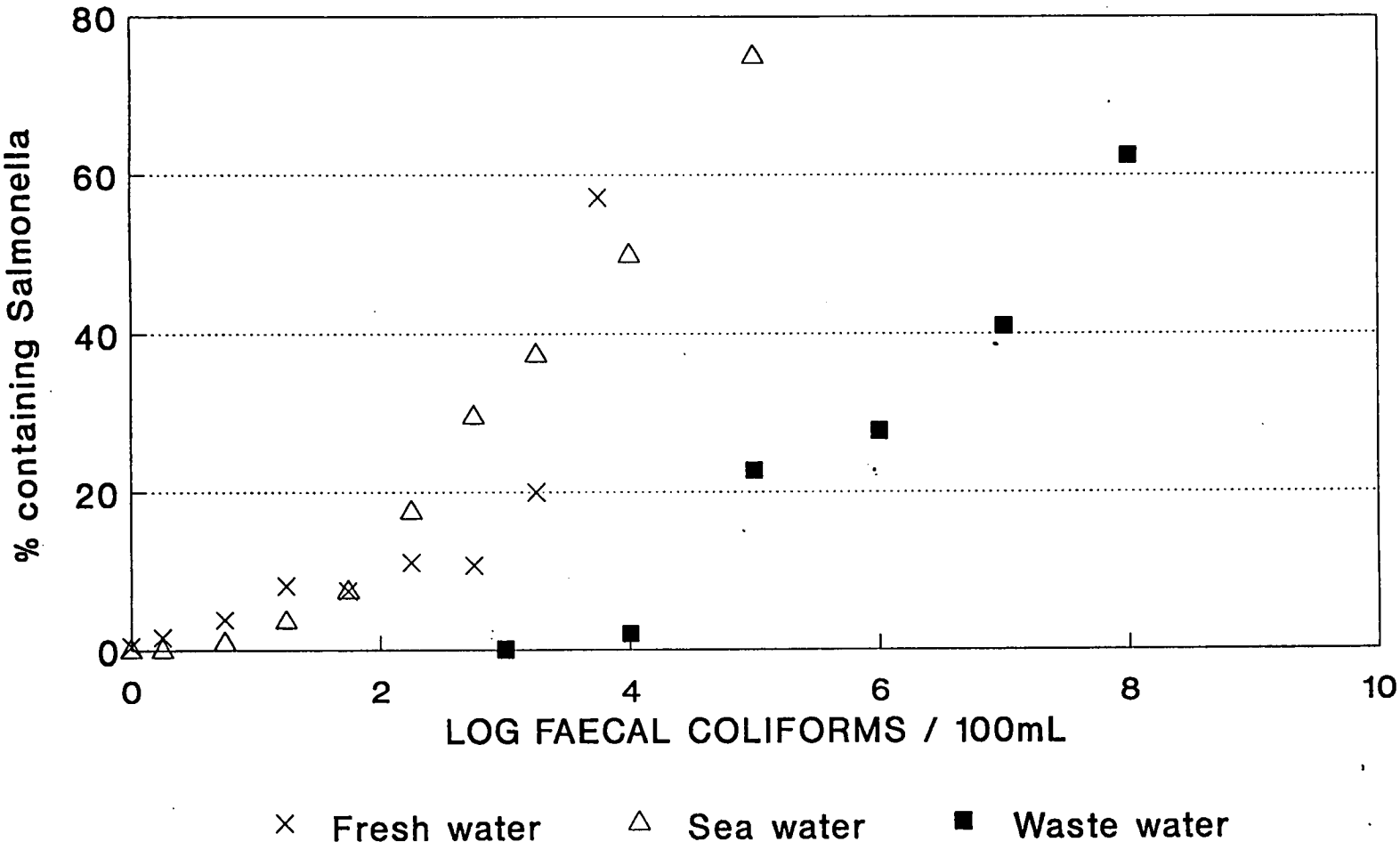
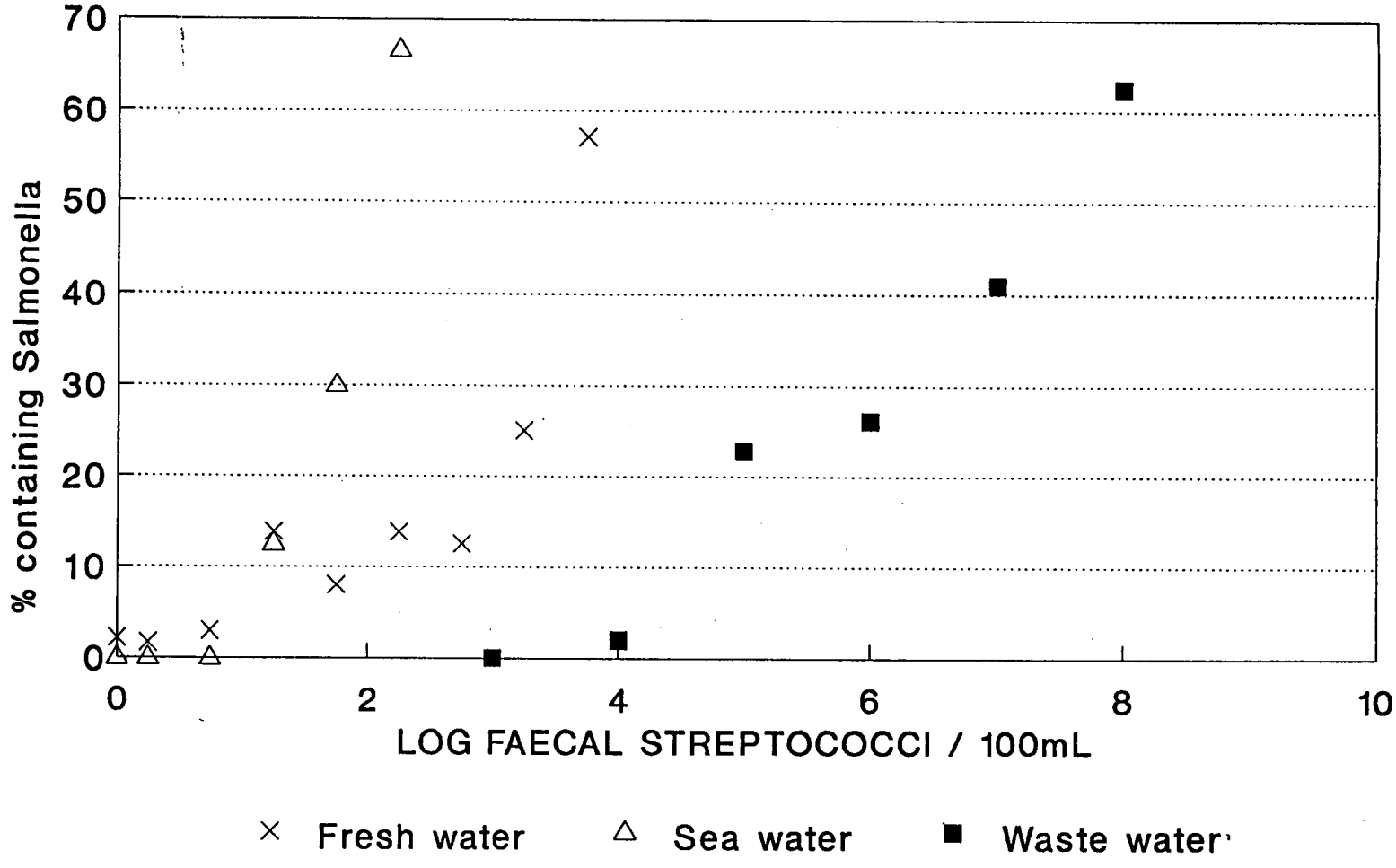


FIG. 28 RELATIONSHIP BETWEEN *SALMONELLA* AND FAECAL STREPTOCOCCI IN FRESH WATER, SEA WATER AND WASTEWATER



PUBLIC HEALTH IMPLICATIONS AND RECOMMENDATIONS

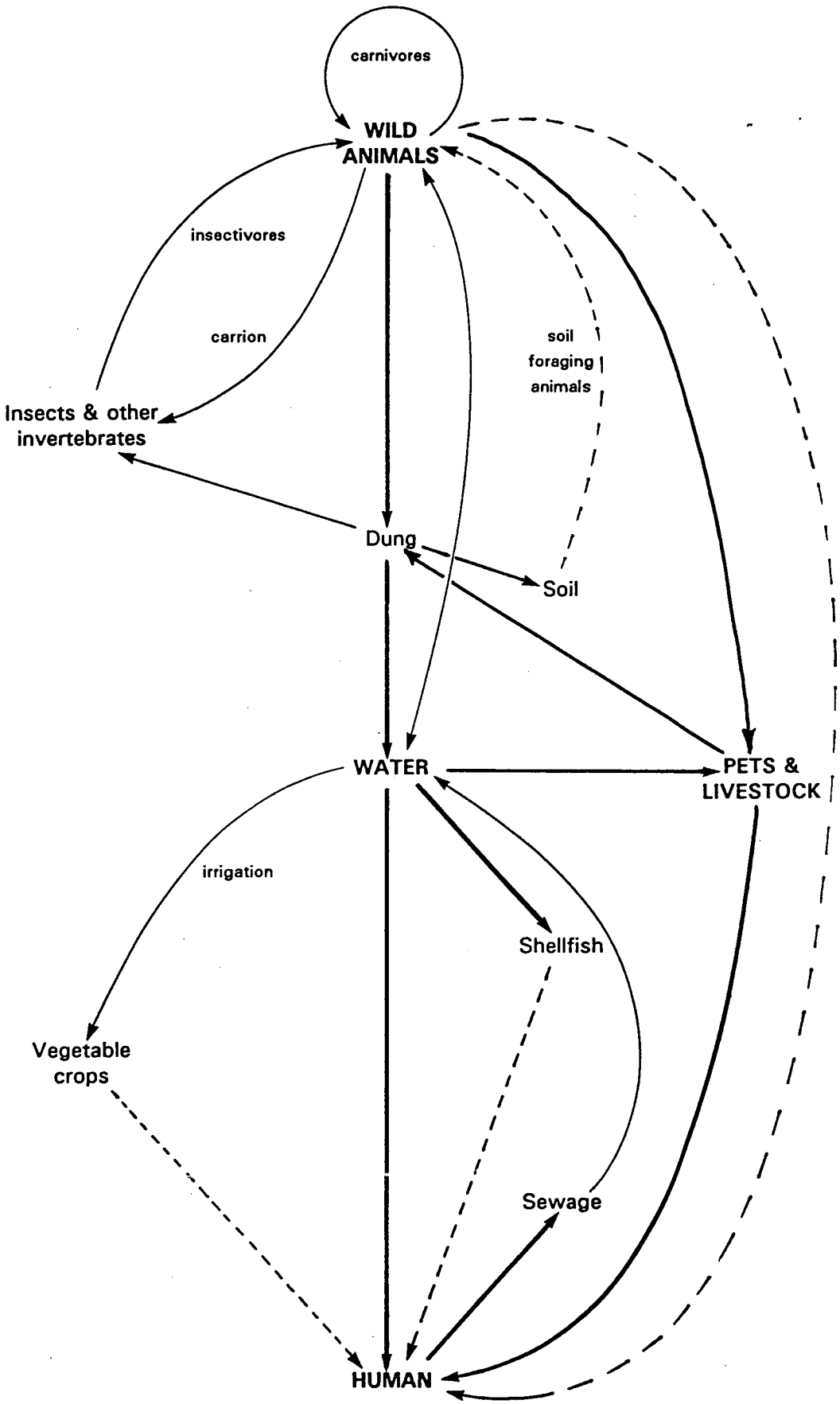
It is most probable that contaminated drinking water is the main source of *Salmonella mississippi* in humans in Tasmania. No salmonellae have been isolated from reticulated water which has been filtered and chlorinated effectively. The absence of bacterial indicators of faecal pollution in water does not preclude *Salmonella* as two instances of water of potable quality containing *Salmonella* have been encountered. Untreated water must be regarded as unsuitable for drinking, even from catchment areas devoid of humans and livestock. The risk of water contamination is highest after heavy rain following a prolonged dry spell.

It is apparent that contaminated meat and poultry was the source of certain *Salmonella* serovars seen in the local human population, in particular *S.sofia*, *S.hadar* and *S.typhimurium*.

Considering approximately one third of human salmonellosis cases in Tasmania are caused by *Salmonella mississippi*, it would seem economically sensible to continue this investigation to determine the mode of transmission from animal to human populations. In addition to contaminated drinking water, infection may be via skinks, either by direct handling or by contact with domestic cats which become infected with *S.mississippi* as a consequence of catching skinks. However the involvement of skinks in human salmonellosis has not yet been proved.

To further the understanding of the epidemiology of *Salmonella mississippi* in Tasmania a survey of *Salmonella* in skinks should be carried out throughout Tasmania and future *S.mississippi* case investigations should establish whether the patient's family or pets had contact with skinks. This will allow the most important pathways of *Salmonella mississippi* infection in Tasmania (Fig.29) to be identified and, where necessary, remedial action taken.

FIG. 29 PROBABLE CYCLING OF *SALMONELLA MISSISSIPPI* IN TASMANIA



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APPENDIX 1

MEDIA RECIPES

The following recipes are prepared with 1000mL a water unless stated otherwise. Dehydrated media (Oxoid, UK) was used if the product was manufactured.

BLOOD AGAR (BA)

Special peptone	23.0g
Starch	1.0g
Sodium chloride	5.0g
Agar	10.0g
pH	7.3 ± 0.2
Sterilisation	121°C / 15min
Additives	50mL defibrinated horse blood

BRAIN HEART INFUSION BROTH (BHIB)

Calf brain infusion solids	12.5g
Beef heart infusion solids	5.0g
Proteose peptone	10.0g
Dextrose	2.0g
Sodium chloride	5.0g
Disodium phosphate	2.5g
pH	7.4 ± 0.2
Sterilisation	121°C / 15min

BUFFERED DILUTION WATER (BDW)

KH_2PO_4	42.5mg
$MgSO_4.7H_2O$	250mg
pH	7.2 ± 0.2
Sterilisation	121°C / 15min

BUFFERED PEPTONE WATER (BPW)

Peptone	10.0g
NaCl	5.0g
Na_2HPO_4	3.5g
KH_2PO_4	1.5g
pH	7.2 ± 0.2
Sterilisation	121°C / 15min

CYSTINE LACTOSE ELECTROLYTE DEFICIENT AGAR (CLED)

Peptone	4.0g
Lab lemco powder	3.0g
Tryptone	4.0g
Lactose	10.0g
L-cysteine	128mg
Bromthymol blue	20mg
Agar N°.1	15.0g
pH	7.3 ± 0.2
Sterilisation	121°C / 15min

DESOXYCHOLATE CITRATE AGAR [HYNES] (DCA)

Lab lemco powder	5.0g
Peptone	5.0g
Lactose	10.0g
Sodium citrate	8.5g
Sodium thiosulphate	5.4g
Ferric citrate	1.0g
Sodium desoxycholate	5.0g
Neutral red	20mg
Agar	12.0g
pH	7.3 ± 0.2
Sterilisation	boil to dissolve

DIAGNOSTIC SENSITIVITY TEST AGAR (DST)

Proteose peptone	10.0g
Veal infusion solids	10.0g
Dextrose	2.0g
Sodium chloride	3.0g
Disodium phosphate	2.0g
Sodium acetate	1.0g
Adenine sulphate	10mg
Guanine hydrochloride	10mg
Uracil	10mg
Xanthine	10mg
Aneurine	20*u*g
Agar	12.0g
pH	7.4 ± 0.2
Sterilisation	121°C / 15min
Additives	70mL defibrinated horse blood

HEKTOEN ENTERIC AGAR (HEA)

Proteose peptone	12.0g
Yeast extract	3.0g
Lactose	12.0g
Sucrose	12.0g
Salicin	2.0g
Bile salts N°.3	9.0g
NaCl	5.0g
Sodium thiosulphate	5.0g
Ferric ammonium citrate	1.5g
Acid fuchsin	0.1g
Bromthymol blue	65mg
Agar	14.0g
pH	7.5 ± 0.2
Sterilisation	boil to dissolve

SELENITE BROTH (SB)

Bacteriological peptone	5.0g
Mannitol	4.0g
Sodium phosphate	10.0g
Sodium biselenite	4.0g
pH	7.1 ± 0.2
Sterilisation	steam for 10 min

MANNITOL SELENITE CYSTINE BROTH (MSCB)

As for Selenite broth with the addition of 0.1mL of L-cystine solution per 10mL immediately before use.

L-CYSTINE SOLUTION

L-cystine	0.1g
NaOH (1M solution)	15mL
Water	85mL
Sterilisation	filter through 0.2*u*m

MEMBRANE ENTEROCOCCUS AGAR (MEA)

Tryptose	20.0g
Yeast extract	5.0g
Dextrose	2.0g
$Na_2HPO_4.2H_2O$	4.0g
Sodium azide	0.4g
Tetrazolium chloride	0.1g
Agar N°.1	10.0g
pH	7.2 ± 0.2
Sterilisation	boil to dissolve

MEMBRANE LAURYL SULPHATE AGAR (MLSA)

Peptone	39.0g
Yeast extract	6.0g
Lactose	30.0g
Phenol red	0.2g
Sodium lauryl sulphate	1.0g
Agar	10.0g
pH	7.4 ± 0.2
Sterilisation	boil to dissolve

MLCB AGAR (MLCB)

Peptone	10.0g
Yeast extract	5.0g
Lab lemco powder	2.0g
Mannitol	3.0g
L-lysine hydrocloride	5.0g
Sodium chloride	4.0g
Sodium tiosulphate	4.0g
Ferric ammonium citrate	1.0g
Crystal violet	10.0mg
Brilliant green	12.5mg
Agar	15.0g
pH	6.8 ± 0.2
Sterilisation	boil to dissolve

NUTRIENT AGAR (NA)

Lab lemco powder	1.0g
Yeast extract	2.0g
Peptone	5.0g
Sodium chloride	5.0g
pH	7.5 ± 0.2
Sterilisation	121°C / 15min

NUTRIENT BROTH (NB)

Lab lemco powder	10.0g
Peptone	10.0g
Sodium chloride	5.0g
pH	7.5 ± 0.2
Sterilisation	121°C / 15min

(Quarter Strength) Ringer Solution (QR)

Sodium chloride	2.25g
Potassium chloride	105mg
calcium chloride.$6H_2O$	120mg
sodium bicarbonate	50mg
pH	7.2 ± 0.2
Sterilisation	121°C / 15min

Rappaports RV Broth (RVB)

Soya peptone	5.0g
Sodium chloride	8.0g
KH_2PO_4	1.6g
$MgCl_2.6H_2O$	40.0g
Malachite green	40mg
pH	5.2 ± 0.2
Sterilisation	115°C / 15min

Urea Agar [Christensen] (UA)

AGAR BASE

Peptone	1.0g
NaCl	5.0g
KH_2PO_4	2.0g
Agar	20g
pH	6.8
Sterilisation	115°C / 20min

ADDITIVES

Add the following filter-sterilised aqueous solutions to the base after cooling to 55°C:

10mL of 10% glucose
6mL of 0.2% phenol red
100mL of 20% urea

XLD AGAR

Yeast extract	3.0g
L-lysine hydrochloride	5.0g
Xylose	3.75g
Lactose	7.5g
Sucrose	7.5g
Sodium desoxycholate	1.0g
Sodium chloride	5.0g
Sodium thiosulphate	6.8g
Ferric ammonium citrate	0.8g
Phenol red	80mg
Agar N°.1	12.5g
pH	7.4 ± 0.2
Sterilisation	boil to dissolve

APPENDIX 2

TABLE A1. LIST OF WILD FAUNA EXAMINED FOR *SALMONELLA*

MAMMALS	
Tasmanian devil	*Sarcophilus harrisii*
tiger cat	*Dasyurus maculatus*
eastern native cat	*Dasyurus viverrinus*
eastern barred bandicoot	*Perameles gunnii*
southern brown bandicoot	*Isoodon obesulus*
echidna	*Tachyglossus aculeatus*
wombat	*Vombatus ursinus*
common ringtail possum	*Pseudocheirus peregrinus*
brushtail possum	*Trichosurus vulpecula*
Tasmanian bettong	*Bettongia gaimardi*
Tasmanian pademelon	*Thylogale billardierii*
Bennett's wallaby	*Macropus rufogriseus*
black rat	*Rattus rattus*
rabbit	*Oryctolagus cuniculus*
feral cat	*Felis catus*
BIRDS	
forest raven	*Corvus tasmanicus*
New Holland honeyeater	*Phylidonyris novaehollandiae*
Australian magpie	*Gymnorhira tibicen*
kookaburra	*Dacelo novaeguineae*
Tasmanian native hen	*Gallinula mortierii*
kelp gull	*Larus dominicanus*
AMPHIBIANS	
common eastern froglet	*Ranidella signifera*
southern toadlet	*Pseudophryne semiarmorata*
spotted grass frog	*Limnodynastes tasmaniensis*
brown tree frog	*Litoria ewingii*
REPTILES	
tiger snake	*Notechis ater*
mountain dragon	*Tympanocryptis diemensis*
blue-tongued lizard	*Tiliqua nigrolutea*
ocellated skink	*Niveoscincus ocellatus*
metallic skink	*Niveoscincus metallicus*
northern snow skink	*Niveoscincus greeni*
MOLLUSCS	
oyster	*Crassostrea gigas*
common garden snail	*Helix aspersa*
INSECTS	
scarab beetle	*Aphodius* sp.
corbie grub	*Oncopera* sp.

APPENDIX 3

TABLE A2. *SALMONELLA* SEROVARS ISOLATED FROM HUMANS IN TASMANIA

YEAR (19__)	80	81	82	83	84	85	86	87	88	89	90
aberdeen				1							
abony										2	
adelaide		1		11	3		1			1	
agona						1			1	4	
anatum		1		1			1	1		1	
*Ar.*61:l,v:1,5,7,z_{57}								1	1		
*Ar.*61:-:-									1		
bahrenfeld	1										
bareilly										1	
birkenhead	1	1	2			1	2		4		
blockey								1	1		4
bovismorbificans	2	2			4	1		2	2	8	5
bredeney		2	1								
cerro							6			2	1
chester						1		1		1	
derby				1						3	1
duesseldorf											1
eastbourne										1	
enteritidis	2		2							3	5
give		3						1	2		
hadar							1			1	7
havana			2	2		1					
heidelberg					1						
infantis			1	1	1		1	1	1	1	
java											1
javiana										1	
johannesburg											3
kentucky										1	
kottbus										1	
krefeld			1								
litchfield			2								
livingstone						1					2
manhattan									1		
mbdandaka								1			
meleagridis		1									
merseyside					1						
mississippi	9	11	26	35	19	23	11	29	64	49	52
montevideo							1				
muenchen								6	4	1	
muenster					1						
newport	2	3	1			1	1				
ohio			1		4	1					
oranienberg						1			1		
paratyphi A	1										1
paratyphi B	1										
potsdam	1								2		

TABLE A2. (CONTINUED)

YEAR (19__)	80	81	82	83	84	85	86	87	88	89	90
saintpaul	4	1	1	1	1		1	1		1	1
seremban									1		
singapore						1		3		1	
sofia II											1
stanley										1	2
taksony										1	
tennessee								1	1		5
thompson							1				
typhi	2							4			
typhimurium	31	23	19	103	39	35	29	79	26	75	39
victoria								1	1	2	2
virchow			1			2	1				2
warragul				1						1	
waycross									2	1	

TABLE A3. PHAGE TYPES OF *S. TYPHIMURIUM* ISOLATED FROM HUMANS IN TASMANIA

YEAR (19__) PT	80	81	82	83	84	85	86	87	88	89	90
1											1
2											3
4	4	4	5	3		2		1			1
5			1	5	1	2					
6		1			1	2	1	2	1	2	1
8	1			1	2					4	
9		1		3	1		3	1	5	20	8
12	1										
12a	2	2				2	2	47	2	6	4
13							1				
14									1		
16									1		
20		1						1		1	
21	1										
22								1			
25				2							
26							1				
27				1	1						
29					2			2			
41				1							
44	1			1	1					4	
55						1		1			
68	1						1			1	
72						1					
90			1	1						1	
101	3	1		6	3	7	8	7		1	
102					2						
104	1										
108						1		1			1
135	4	6	6	65	17	8	5	5	9	24	9
141	7	3	2	9	8	6	4	2	2	3	
145									2		2
154							1				
170									1		2
179	2	1		3	1			8	1	3	
202							1				
RDNC	1			1		3	1			4	7
untypable	3	3	4	1					1	1	

APPENDIX 4

TABLE A4. *SALMONELLA* SEROVARS ISOLATED FROM HUMANS IN AUSTRALIA

YEAR (19__)	80	81	82	83	84	85	86	87	88	89	90
4,12:d:-	23	53	10	19	14	22	10	9	4	8	5
abaetetuba						1	1				
aberdeen	6	8	19	12	17	22	32	26	37	43	43
abony	21	14	36	26	9	15	18	34	10	33	9
adelaide	61	112	76	63	59	74	47	47	6	102	89
agodi											1
agona	42	49	73	69	79	45	45	74	41	50	40
alachua	5	21	2		1	1			4	4	1
albany	1		1	1	1			1	4	2	2
alsterdorf-II			2			1		1			
Amsterdam								1	7		3
Anatum	182	257	185	155	119	99	121	146	224	157	161
angoda	1			1				1			
ank											1
arechavaleta					1						
augustenborg						1					
bahrenfeld	11	9	9	4	3	6	5	5	7	2	8
ball	18	17	17	15	15	13	23	11	7	13	12
bardo		1									1
bareilly	6	13	8	9	8	4	3	3	6	7	3
bere	2										
bergedorf										1	1
berta	2							2	4	27	5
binza	1			5	1						
birkenhead	45	62	76	63	64	59	88	124	137	106	145
bleadon		1		1	1		1		1	2	
blegdam								1			
blockey	11	9	19	49	15	18	15	35	34	41	24
blukwa		4								2	
bonariensis										1	
bonn		10	6	3	3	2	2		2		1
bootle		1		1	2		3	1			1
bornum	1				1						
bournemouth	2			1		3					
Bovismorb	401	255	124	207	134	138	115	94	145	243	221
braenderup	1	15	3	3	4	2	7	6	5	2	2
brandenberg					1	1			5	1	
bredeney	55	35	36	35	24	21	42	21	14	21	30
breuleken	2		4		2	2	2		3	2	
brisbane		1	5	3	1		2		1		1
bronx				1						1	
broughton	1					1	1	1			2
brunei			1	1						1	
bukavu	6	5	4	2	1	1	2	1	2	2	1

TABLE A4. (CONT'D)

YEAR (19__)	80	81	82	83	84	85	86	87	88	89	90
butatan					1						
cambridge					1						
cannonhill			1								
cerro	14	4	6	3	1	3	104	26	42	58	68
chailey										3	2
champaign	3		1	1	1			2		1	
charity	3		4	6	2	3	2		4		2
chester	131	223	209	182	118	147	167	184	177	138	149
chicago					1						
Choleraesuis	2	5	2	5	1	2		2	9	2	4
coleypark	2	1	1								
corvallis	1										
cubana	8	2	1	1		2	6	4	4	6	
dan									1		
decatur			1		4	1		2	4	2	
denver									1		
derby	271	201	808	67	78	42	40	39	41	51	47
djugu										1	
drypool		2									
dublin	7	4	7	2	6	3	7	1	14	10	11
duesseldorf											1
eastbourne	21	22	15	37	31	14	57	25	31	26	26
eimsbuettel	2	21	9	7	1						
elizabethville		1									
emek	1	6	8	5	4	5	4	4	7	6	2
emmastad	1	3	2	2	2	4		1	3	1	1
Enteritidis	70	76	91	78	58	59	55	88	78	116	81
fitzroy		1		1	1						
foulpointe				1							
fremantle II	1	25	5	4		3	4	25	4	7	6
galiema		1						1			
gaminara		4	1					4	2		1
gatineau				1							
gatuni		1						1			
Give	82	74	155	75	17	34	26	74	70	27	20
goldcoast										1	
grabouw				1							
haardt							1		9	4	2
hadar			3			2	9		18	39	25
haifa	1	5	1		5	2	2	5	3	1	2
havana	159	200	192	154	135	144	124	200	99	58	105
heidelberg	8	10	4	4	25	49	171	10	270	114	128
heron								1			
Hessarek		5	7	1	5	5	2	5	4	5	1
hindmarsh					1					1	
hofit			1								
horsham							1				
houten IV	3	24	12		1	1	3	24		1	3
hull						1					
hvittingfoss	13	13	12	14	16	19	12	13	17	16	13
idikan										2	5

TABLE A4. (CONT'D)

YEAR (19__)	80	81	82	83	84	85	86	87	88	89	90
indiana	4	2		2	5	1	2	2	3	1	
infantis	210	188	182	103	69	82	76	188	135	179	139
irumu		1		2		1		1	1		
isangi	1	1						1		1	
israel										1	
jangwani	4	3	5	8	9	6	3	3	3	3	
Java	33	46	37	45	72	41	67	46	56	46	44
javiana	8	9	9	10	7	7	7	9	10	11	8
johannesburg	6	51	9	5	2	1	10	51	12	14	18
kaapstad					1						
kentucky	2	3	1		4	3	2	3	6	8	8
kiambu										1	
kimberley	1	2	2	2		2	3	2	2		2
kinondoni	1	15	2		6	1	5	3	2	1	5
kottbus	12	57	23	43	18	14	32	27	23	27	35
krefeld	10	4	10	1	4	3	3	11	5	3	2
lagos									1		
lanka		1		1							
lansing	22	32	23	48	22	28	26	25	24	30	28
lavana				1							
Lexington	3	4	4	3	5	1	1	6	2	6	
lille	1		3	1			1		1	1	
lindenburg										1	
lindern									1	4	1
litchfield	39	40	29	29	35	34	40	57	51	50	34
liverpool										2	7
Livingstone	5	3	1	3	7	6	2	5	8	2	3
lohbruegge IV	1	1	1				1		1	1	
lomita						2					
London	5	1	8	3	5		1	2	5	2	3
manhattan		1		2				1	2		
mbdandaka	19	3	5	5	15	10	4	5	12	11	18
Meleagridis	5	3	7	8	8	2	1	6	3	4	3
mendoza		1									
merseyside					1						
mgulani				2	1	1	2	7	11	23	24
mikawasima			3						2	1	
mississippi	10	14	34	39	22	25	14	31	67	60	54
mobeni			1								
molade									1		
monschaui		1	1			1					
montevideo	6	10	12	3	7	12	6	10	13	8	15
morotai										1	
muenchen	157	217	285	186	163	138	127	141	184	174	147
muenster	2	1			2	1				1	
neinstedten	2	7		4	1						
neukolln		1									
newbrunswick	2	14	4	3	2			1			

TABLE A4. (CONT'D)

YEAR (19__)	80	81	82	83	84	85	86	87	88	89	90
newington	9	10	26	18	20		1	2			
newlands										1	
newport	76	512	44	76	28	45	53	28	21	25	46
newyork								1			
Ohio	15	63	42	17	27	27	10	8	13	7	15
ohlstedt	4	6	2	6	3	12	3	6	6	11	6
omderman	1										
onderspoort	8	5	3	9	4	3	4	9	7	3	9
oranienberg	64	82	51	51	66	60	42	71	66	112	62
orientalis	34	16	13	9	6	7	7	15	7	48	36
orion	25	29	26	37	42	34	38	19	28	29	41
oslo	5	13	8	14	4	2	3	8	13	4	3
panama	3	3	6	6	11	3	5	5	8	5	2
Paratyphi A	12	14	13	14	22	15	6	21	7	23	4
Paratyphi B	13	10	11	4	17	8	3	7	18	6	7
paratyphi C		1				2					
plymouth			1								
poona		10	1	3	9		3	12	10	5	14
portsmouth		1									
potsdam	13	41	31	20	51	47	40	43	134	45	44
pullorum					2	1					
quinhon				1							
ramatgen	1		1			1	1		2		
raus		1									
reading		10	6	1	6	6	6	7	3	33	17
richmond	1	1	1		2		3	1			
rissen								1		4	
rottnest							1	1			
rubislaw	20	27	13	10	18	34	13	18	20	21	16
sachsenwald				2	5		3		2	2	1
Saintpaul	254	213	178	201	193	227	217	286	221	200	219
salford											1
sandiego		2			1			3			2
schwarzengrund		42	37	11	4	4	10	3	17	8	4
Senftenberg	54	158	81	45	50	42	54	46	38	41	42
seremban									1		
singapore	88	135	105	115	290	77	68	67	110	129	51
Sofia II	3	13	5	7	8	7	11	16	17	21	18
souza		1									
stanley	4	5	9	19	29	10	3	81	20	25	13
stanleyville		1						1	2		
staoveli	1					1					
sunnycove										2	
sylvania							3				
taksony		1								1	
tennessee	30	42	29	45	31	29	42	44	29	33	44
thielallee	1										
thompson	9	8	8	20	11	16	6	17	17	20	9
toowons											1

TABLE A4. (CONT'D)

YEAR (19__)	80	81	82	83	84	85	86	87	88	89	90
tounouma										1	
treforest		2	3	2	2		1	1	5	1	
tshiogive		1									
Typhi	65	68	66	27	98	40	14	46	30	59	11
Typhimurium	3601	3333	1293	1956	1378	1888	1364	1462	1737	2403	2029
uganda				1	1			1		1	1
ullevi											1
uphill	2										
urbana	3	14	15	6	17	11	10	13	7	6	4
vejle											1
victoria	1	12	8	1		5	2	1	2	3	2
virchow	106	181	248	310	203	245	257	261	308	244	267
virginia						1	1			1	
wandsbek II	7	38	5	15	8	5	7	7	6	4	9
wandsworth	39	52	14	21	12	18	25	32	13	27	14
wangata									1	1	1
warragul	1	4	7	1						2	
waycross	20	38	32	22	18	20	21	45	49	41	42
welikade	13	7	18	19	21	32	30	42	26	9	24
weltevreden	29	39	28	39	42	15	24	21	32	17	17
westhampton										1	
worthington	5	3	1	7	1	3	3	2	1	2	1
yarrabah			1					1	1		
Zanzibar	1	5	6	12	6	3	4	7	10	11	15
zehlendorf	2			1	2	1	1	2	1		2

APPENDIX 5

TABLE A5. ANNUAL DISTRIBUTION OF *SALMONELLA MISSISSIPPI* IN GEOGRAPHIC AREAS OF TASMANIA

	YEAR (19__)	80	81	82	83	84	85	86	87	88	89	90
A	Hobart	1	4	7	5	5	5	4	5	7	11	10
B	Southeast					1	1				1	2
C	S. midlands		1	1	1						1	
D	Channel	1	1		1	1			3	2		1
E	Huon	1			1	1						
F	Derwent			1			1	1			1	
G	E. coast	2		1	1	1		2		1		
H	N. midlands		1						2	2	4	3
I	Launceston	1	2	4	1	1			1	5	6	8
J	Northeast	2	1	1	1		3		1	3	1	4
K	Flinders Is.			1			1					
L	King Is.											
M	W. Tamar			1		1				1	1	
N	Mersey	1	1	1	5	2	5	3	9	28	16	14
P	Burnie			3	2	1					2	2
Q	Northwest				3	1			1			
R	W. coast			1		2				1		
S	Plateau											
	LGH											1
	RHH				1							1
	NWGH				1	2						
	MGH											
	HP										1	
	LP			1	1		1				1	1
	NWP											
	PHL				2							
TOTAL		9	11	24	26	19	17	10	22	50	46	47

TABLE A6. ANNUAL DISTRIBUTION OF *SALMONELLA* SPP. IN GEOGRAPHIC AREAS OF TASMANIA

	YEAR (19__)	80	81	82	83	84	85	86	87	88	89	90
A	Hobart	7	12	23	45	17	16	17	36	18	36	26
B	Southeast				4	1	2	5			4	3
C	S. midlands	3	2	1	6	4		2	1		4	1
D	Channel	1	1	1	3	5	3	3	6	2	2	5
E	Huon	1	1		1	2		1			3	1
F	Derwent			3	2	1	2	2			2	1
G	E. coast	2		2	2	2		2	1	1	1	
H	N. midlands	3	3	1	2	4	6	4	25	8	16	15
I	Launceston	6	6	10	9	9	9	7	18	18	25	25
J	Northeast	4	1	2	3	4	7	1	5	4	6	7
K	Flinders Is.	1	1	1	1	1	1					3
L	King Is.				1		2				4	5
M	W. Tamar	4	2	1	2	4	2		2	2	3	
N	Mersey	3	2	4	9	8	8	5	15	37	33	19
P	Burnie		2	4	4	4	4	1	3	2	9	6
Q	Northwest				5	1	1	1	4			
R	W. coast		1	3	1	3			2	1		5
S	Plateau											
	LGH	1	1		1					1		3
	RHH	3	1	1	9	2	3	1	2			1
	NWGH	7	2	2	4	7		1				
	MGH	1			1						3	
	HP	6	6	1				2			1	
	LP	2	2	1	4		1		1		5	6
	NWP				1		1					
	PHL								1			
TOTAL		55	46	61	120	79	68	56	122	93	157	132

APPENDIX 6

NAME OF DISEASE

EPIDEMIOLOGICAL RECORD SHEET AND
PROCEDURE FOR INVESTIGATING GASTROINTESTINAL DISEASES

This form is to be completed when making retrospective investigation of a bacteriologically confirmed case of Gastrointestinal Disease.

Circle Yes/No where applicable.

FULL NAME OF PATIENT ..... AGE ...

ADDRESS ..... TELEPHONE (Home) .....

OCCUPATION .....

NAME & ADDRESS OF EMPLOYER .....

..... TELEPHONE (Work) .....

CONTACTS (Note if similarly affected)

CLOSE (Full Name, Age & Address) .....
.....
.....
.....

REMOTE (Full Name, Age & Address).....
.....
.....
.....

Does any household member work as a food handler or care for small children?

Yes No If yes, specify who. Also state name and address of employer.

DATE OF NOTIFICATION:	SPECIMENS TAKEN FOR LAB TEST			RESULT
	Suspected food:	Yes	No	
	Vomit:	Yes	No	
	Faeces:	Yes	No	
	Blood:	Yes	No	
	Others: Give details			

DISEASE NOTIFIED:

SEROTYPE:

SYMPTOMS:			SYMPTOMS:		
Fever:	Yes	No	Constipation:	Yes	No
Nausea:	Yes	No	Headache:	Yes	No
Vomiting:	Yes	No	Rash:	Yes	No
Abdominal Cramps:	Yes	No	Joint Pain:	Yes	No
Diarrhoea:	Yes	No	Neurological		
Bloody Diarrhoea:	Yes	No	Symptoms:	Yes	No

Was Doctor consulted? Yes No Was patient Hospitalized? Yes No
If yes, complete below:- If yes, complete below:-

Name: Telephone: Hospital:
Address: Dates: Admission Discharge

NON-HOUSEHOLD EXPOSURE:

Exposure to any person ill with diarrhoea or fever in the period of a week prior to onset of symptoms. Yes No If yes see below.

Name of Person(s)	Address	Telephone	Contact Date
.....			
.....			
.....			
.....			

TRAVELLING AWAY FROM HOME: Where: Date:

Yes No

WATER EXPOSURE:

Source of home supply: Public (Treatment Details) Private (Give Details)

Swim or participate in water sports in the week before onset.-

YesNo (give details)

FOOD INTAKE WITHIN 72 HOURS OF ONSET OF ILLNESS

A. SOURCE OF FOODS

1. Foods eaten at home:

   Details:

   Where obtained

2. FOODS FROM TAKE-AWAY COOKED FOOD SHOPS

   Give details:

   Where obtained:

3. FOOD EATEN AT RESTAURANTS, CANTEENS CAFETERIAS ETC

   Give details:

   Where obtained:

   Is there a need to follow up with an inspection of premises if suspected or implicated? Yes No

B. DETAILS OF FOOD EATEN

1. MILK

	Packed On/Use by date:	
Raw:	Yes	No
Pasteurised:	Yes	No
Goat:	Yes	No
Powdered/Baby Formula:	Yes	No
Other: Give details:		

   Brand details:

2. MILK PRODUCTS

	Packed On/Use by date:	
Raw:	Yes	No
Pasteurised:	Yes	No
Butter:	Yes	No
Cream:	Yes	No
Yoghurt:	Yes	No
Cottage Cheese:	Yes	No
Cheeses:	Yes	No

   Other: Give details:

   Brand details:

3. EGGS

Hen:	Yes	No
Duck:	Yes	No
Cracked:	Yes	No
Other: Give details		

4. SWEETS, CAKES, MEAT PIES ETC.

Sweets made with eggs and/or milk:	Yes	No
Cakes filled/coated with cream:	Yes	No
Meat pies etc.	Yes	No

If yes, give details.

5. Dressings, sauces, pre-prepared salads, coleslaws etc. Yes No
If yes, give details).

6. MEATS ETC. (give details : frozen, chilled, pre packed or fresh)

Packed On/Use by date

Poultry	Yes	No
Minced Meat:	Yes	No
Sausage:	Yes	No
Ham:	Yes	No
Pate:	Yes	No
Dry Cured:	Yes	No
(Salami type):	Yes	No
Beef:	Yes	No
Veal:	Yes	No
Pork:	Yes	No
Lamb:	Yes	No
Rabbit:	Yes	No

Other - specify:

Cuts: Give details:
If eaten raw or rare, give details:

7. VEGETABLES

VEGETABLES	HOME/GARDEN	ORGANICALLY GROWN	SHOP
Mushrooms			
Zucchini/cucumber			
Lettuce			
Cabbage			
Carrot			
Celery			
Capsicum			
Tomato			
Raddish			
Herbs			
Sprouts			
Other salad veg			

8. SEAFOOD

Fish (Fresh Water)	Yes	No
Fish (Marine)	Yes	No
Shellfish	Yes	No
Fresh	Yes	No
Frozen	Yes	No

9. OTHER FOODS CONSIDERED BY PATIENT LIKELY TO HAVE CAUSED ILLNESS

Give details:

Where obtained:

HOME ENVIRONMENT

Pets: (Type) Petfood:

Vermin: Pet Faecal samples Yes No

Exposure to other animals: Result

Sewage disposal: Method:

Sewage Overflow Backflow etc (give details):

Difficulties with septic tank effluent disposal (give details):

MEDICATION

Medication Yes No (Give details):

Medication started pre infection Yes No

Medication started post infection Yes No

ANY PRE-EXISTING MEDICAL CONDITION

INVESTIGATORS COMMENTS:

A. HOME ENVIRONMENT

B. SUSPECTED SOURCE

C. OTHER

Investigator: Name:
Signature: .....
Date: .....
Phone Number: .....

Please send form when completed to:

Director of Public Health