

## Animal salmonella surveillance in Peninsular Malaysia, 1981-1985

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### SUMMARY

During the 5-year (1981-5) surveillance period, 2322 salmonella isolations were recorded from animals and other non-human sources in Peninsular Malaysia. This was an increase of 356% over the preceding 5-year period. The 83 serotypes isolated were recovered from 41 sources. Of these 34 were new serotypes bringing the total number of serotypes isolated from non-human sources to date up 97. Food animals and edible animal products accounted for 92.2% of the total isolations, with cattle and beef accounting for 70% of the total. *Salmonella dublin* was the most frequently isolated serotype, whereas *S. typhimurium* had the widest zoological distribution. More than 80% of the non-human salmonella serotypes have also been reported in man in this country.

### INTRODUCTION

Salmonellosis remains a persistent threat to human and animal health in spite of the advances made in its detection and control. In the livestock and poultry industries it causes losses through the death of young animals, abortions, unthriftiness, decreased milk, meat and egg production, expensive testing and control programmes, and reduced value of contaminated products (Houston, 1984).

Salmonella isolations from animals in Peninsular Malaysia were first made at the Veterinary Research Institute (VRI), Ipoh in 1954, when *Salmonella choleraesuis* and *S. typhimurium* were isolated from pigs (Wells, 1955). These were followed by isolations of a number of serotypes from pigs, sheep, rats and guinea-pigs at the Institute for Medical Research (IMR), Kuala Lumpur (Bhagwan Singh, 1955).

To assist in the study of the epidemiology and control of salmonellosis in animals and in man the VRI in 1971 initiated an Animal Salmonella Surveillance Programme. Joseph (1971) and Joseph *et al.* (1976), Joseph, Anwar & Jegathesan (1978) and Joseph *et al.* (1986) have published periodic surveillance reports up to the year 1980. These reports provide useful information on the changing patterns of salmonellosis in animals. There has been a steady increase over the years in the number of salmonella serotypes isolated and in the number of animal species involved.

This report records the animal isolations and the zoological distribution of all the salmonella serotypes isolated and/or serotyped at the VRI, the Regional

Table 1. Frequency and zoological distribution of salmonella serotypes from non-human sources, 1981-5

Group and serial no.	Serotype	Source and no. of isolations								Total	
		1	2	3	4	5	6	7	8		
B	1	<i>paratyphi B</i>	—	—	—	—	—	2	1	—	3
	2	<i>paratyphi B</i> var. <i>java</i>	25	2	2	—	1	—	2	—	32
	3	<i>sofa</i>	2	1	—	114	1	—	—	16	134
	4	<i>stanley</i>	11	—	—	2	7	2	1	—	23
	5	<i>schwarzengrund</i>	3	—	—	—	1	—	—	—	4
	6	<i>saintpaul</i>	2	—	—	—	3	—	—	—	5
	7	* <i>chester</i>	—	—	—	4	—	—	—	10	14
	8	<i>derby</i>	—	—	2	1	1	2	—	—	6
	9	<i>agona</i>	1	—	1	5	3	—	—	—	10
	10	<i>typhimurium</i>	459	11	3	17	1	1	7	3	502
	11	<i>typhimurium</i> var. <i>copenhagen</i>	5	—	—	1	—	—	1	—	7
	12	<i>heidelberg</i>	1	1	—	4	2	1	—	—	9
	13	<i>haifa</i>	—	—	—	5	—	—	—	—	5
	14	group B (untypable)	—	—	—	3	—	—	—	—	3
C1	15	<i>oslo</i>	1	—	—	—	—	—	—	—	1
	16	<i>ohio</i>	—	—	—	1	2	—	—	9	12
	17	<i>chloeraesuis</i> var. <i>kunzendorf</i>	—	—	34	—	—	—	—	—	34
	18	* <i>kisii</i>	—	1	—	—	—	—	—	—	1
	19	* <i>isangi</i>	—	—	—	—	8	—	—	1	9
	20	* <i>livingstone</i>	—	—	—	1	—	—	—	—	1
	21	* <i>larochelle</i>	2	—	—	—	1	—	—	—	3
	22	* <i>lomita</i>	—	—	1	—	—	—	—	—	1
	23	<i>braenderup</i>	—	—	—	7	—	—	—	2	9
	24	<i>montevideo</i>	—	—	—	3	1	—	—	—	4
	25	<i>augustenburg</i>	—	—	—	—	2	1	—	1	4
	26	<i>thompson</i>	—	—	—	1	—	—	—	—	1
	27	* <i>singapore</i>	—	—	—	—	—	—	—	1	1
	28	* <i>escanaba</i>	1	—	—	—	—	—	—	—	1
	29	* <i>concord</i>	—	—	—	—	1	—	—	—	1
	30	<i>virchow</i>	3	2	—	1	6	—	—	—	12
	31	<i>infantis</i>	1	—	—	2	—	—	—	8	11
32	<i>bareilly</i>	—	—	—	11	3	4	—	3	21	
33	* <i>inganda</i>	—	—	—	—	—	1	—	—	1	
34	<i>mbandaka</i>	3	—	—	6	1	—	—	—	10	
35	<i>tennessee</i>	—	—	—	—	—	—	—	2	2	
C2	36	* <i>muenchen</i>	—	—	—	—	1	—	—	—	1
	37	<i>newport</i>	5	1	—	—	6	—	—	1	13
	38	<i>blockley</i>	3	—	5	27	17	—	2	8	62
	39	<i>litchfield</i>	21	1	—	—	—	—	—	—	22
	40	<i>bovis-morbificans</i>	3	—	—	2	1	2	—	—	8
	41	* <i>hadar</i>	1	—	—	1	9	—	—	—	11
C3	42	<i>emek</i>	2	—	—	18	1	—	—	22	43
	43	<i>kentucky</i>	17	—	—	60	2	1	—	11	91
	44	* <i>hindmarsh</i>	—	—	—	—	—	—	3	—	3
	45	<i>albany</i>	—	—	—	—	1	—	—	—	1

Table 1 (cont.)

Group and serial no.	Serotype	Source and no. of isolations								Total	
		1	2	3	4	5	6	7	8		
D1	46	<i>enteritidis</i>	—	—	—	4	—	1	—	—	5
	47	<i>dublin</i>	852	13	—	—	—	—	—	3	868
	48	<i>javiana</i>	38	1	—	3	3	—	—	—	45
E1	49	<i>anatum</i>	4	—	—	3	6	1	—	1	15
	50	<i>meleagridis</i>	—	—	—	4	2	—	—	—	6
	51	<i>*nchanga</i>	—	—	—	—	8	—	—	—	8
	52	<i>london</i>	1	—	—	1	1	—	—	—	3
	53	<i>give</i>	—	—	—	—	—	1	—	—	1
	54	<i>weltevreden</i>	57	4	1	7	8	3	11	6	97
	55	<i>lexington</i>	—	—	—	—	4	—	1	4	9
E2	56	<i>lanka</i>	—	—	—	—	—	—	—	3	3
E4	57	<i>*liverpool</i>	—	—	—	—	1	—	—	—	1
	58	<i>senftenberg</i>	6	—	—	3	9	—	—	—	18
	59	<i>*krefeld</i>	—	—	2	2	—	—	—	—	4
F	60	<i>*luciana</i>	—	—	—	—	1	—	—	—	1
	61	<i>chingola</i>	6	—	—	—	—	—	—	—	6
G1	62	<i>*raus</i>	1	—	—	—	—	—	—	—	1
	63	<i>poona</i>	—	—	—	—	1	—	—	—	1
G2	64	<i>havana</i>	4	—	—	1	—	—	—	—	5
	65	<i>*cubana</i>	2	—	—	—	—	—	—	—	2
I	66	<i>hvitlingfoss</i>	9	—	—	—	7	—	1	—	17
	67	<i>*weston</i>	—	—	—	—	1	—	—	—	1
	68	<i>*orientalis</i>	1	—	—	—	—	—	—	—	1
K	69	<i>*cerro</i>	—	—	—	—	1	—	—	—	1
N	70	<i>urbana</i>	4	—	—	—	—	—	—	—	4
	71	<i>matopeni</i>	—	—	—	—	—	—	1	—	1
	72	<i>*kumasi</i>	2	—	—	—	1	—	—	—	3
O	73	<i>*adelaide</i>	11	—	—	—	—	—	—	—	11
	74	<i>*alachua</i>	—	—	—	—	—	—	—	1	1
P	75	<i>*mgulani</i>	—	—	—	—	5	1	—	—	6
Q	76	<i>wandsworth</i>	—	—	—	—	6	—	—	—	6
T	77	<i>*fremantle</i>	—	—	—	—	1	—	—	—	1
	78	<i>*toricada</i>	—	—	—	—	—	—	2	—	2
	79	group T (untypable)	—	—	—	1	—	—	—	—	1
U	80	<i>*arizonae</i> (Ar:21:1,2,5:-)	—	—	1	—	—	—	—	—	1
	81	<i>*houten</i>	—	—	1	1	—	1	—	—	3
V	82	<i>*lohbruegge</i>	—	—	—	—	—	—	2	—	2
X	83	<i>*bere/tabligbo</i>	1	—	—	—	—	—	—	—	1
	84	group X (untypable)	—	—	—	—	—	—	—	6	6
61	85	<i>*arizonae</i> (Ar:26:33:28)	—	—	—	—	1	—	—	—	1
	86	<i>*arizonae</i> (Ar:26:23:30 {40a, 40c})	1	—	—	—	—	—	—	—	1
		Total	1572	38	53	327	150	25	35	122	2322

Source code: 1, cattle and buffaloes; 2 goats and sheep; 3, pigs; 4, chickens and ducks; 5, edible animal products; 6, companion and laboratory animals; 7, wild and captive animals; 8, environment and feed samples.

\* First-time isolations from animal sources in Peninsular Malaysia.

Veterinary Diagnostic Laboratories (RVDLs) and the Veterinary Public Health Laboratory (VPHL) in Peninsular Malaysia for the 5-year period 1981–85.

### MATERIALS AND METHODS

Specimens submitted to the VRI and RVDLs comprised carcasses, organs or tissues of dead animals, faecal and aborted materials from live animals, eggs and environment samples from hatcheries and farms. Specimens submitted to the VPHL were largely frozen, chilled and fresh meats, dehydrated soup powders and stocks, freeze-dried foods and milk and milk products.

Unprocessed and raw specimens were cultured directly on to tryptose blood agar (Oxoid) or MacConkey agar (Difco), and into either selenite F (Difco) or tetrathionate brilliant-green bile (Merck/BBL) enrichment broths. For processed specimens (frozen, pre-cooked, freeze-dried, etc.), procedures recommended by the American Public Health Association (Poelma & Silliker, 1976) were followed. This involved the process of overnight pre-enrichment in nutrient broth (Oxoid) before inoculating selective enrichment broths such as selenite F or tetrathionate or both.

Subcultures from enrichment media were made on to selective solid media such as MacConkey agar, brilliant-green sulfadiazine agar (Difco), desoxycholate citrate agar (BBL) and xylose-lysine-desoxycholate agar (BBL). Subcultures were usually made after 24 h incubation at 37 °C and in some cases repeated at 48 h incubation.

Salmonella-like colonies on solid media were inoculated into triple-sugar iron agar (Oxoid) slants, and cultures that gave typical reactions were subjected to confirmatory biochemical and slide agglutination tests with polyvalent O and H salmonella antisera (Wellcome).

Confirmed salmonella isolates were then submitted to the VRI with relevant details as required by the Animal Salmonella Surveillance Programme. At the VRI the isolates were serotyped according to the Kauffmann–White classification scheme using a battery of somatic and flagellar antisera (Wellcome/Difco).

### RESULTS

A total of 2322 salmonella isolates were serotyped during the 5-year surveillance period, 1981–5. Their annual distribution is shown in Fig. 1. Ten isolates were not fully typable; the remaining 2312 isolates were classified into 83 serotypes under 22 groups of the Kauffmann-White classification scheme.

Of the 83 serotypes and 22 groups, 34 and 7 respectively were recorded for the first time from non-human sources in Peninsular Malaysia (Table 1). Table 1 also gives the zoological distribution of the isolates. The isolations were made from 41 non-human sources grouped under (1) cattle and buffaloes, (2) goats and sheep, (3) pigs, (4) chickens and ducks, (5) edible animal products, (6) companion and laboratory animals, (7) wild and captive animals and (8) environment and fecal samples.

Of the total isolates 92.2% were from food animals (cattle, buffaloes, sheep, goats, pigs, chickens and ducks) and edible animal products (beef, mutton, pork

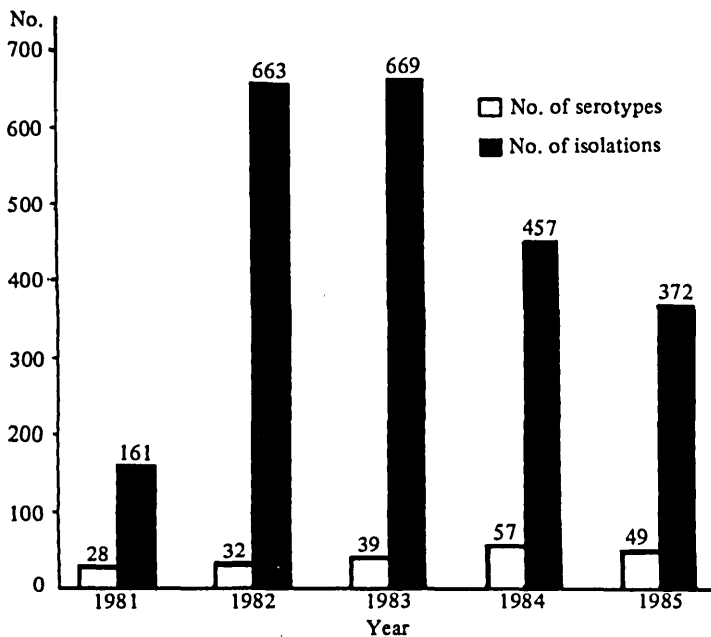


Fig. 1. Annual salmonella isolations and number of serotypes from non-human sources, 1981-5.

Table 2. *Salmonella* isolations from edible animal products 1981-5

	Beef/ buffalo meat	Mutton	Pork	Chicken meat	Eggs	Frogs' legs	Total
No. of samples tested	757	23	111	62	3833	142	4928
No. of samples positive	60	2	11	25	6	46	150
Percentage of samples positive	7.9	8.7	9.9	40.3	0.2	32.4	3.0
No. of salmonella isolations	60	2	11	25	6	46	150
No. of salmonella serotypes	23	2	7	4	5	16	45

chicken meat, eggs and frog legs). Cattle, buffaloes and beef alone accounted for 70% of the total. Isolations from chickens and ducks were next highest. Details of the isolations made from edible animal products are given on Table 2. From 1983 to 1985, 4928 meat and egg samples were tested, and from these 150 salmonella isolations were made, with the highest incidence in chickens and imported frog legs.

Twenty-five salmonella isolations were made from companion (14 isolations) and laboratory (11 isolations) animals (Table 3). Nineteen species of wild and captive birds, mammals and reptiles from local zoos, pet shops and farms were found positive for salmonella (Table 4). A total of 177 isolations were made from the environment and 5 from animal feed samples (Table 5). Of these the vast majority were from hatcheries.

Table 3. *Salmonella* isolations from companion and laboratory animals, 1981—5

No. and animal species	<i>Salmonella</i> serotype (no. of isolations)
8 dogs	<i>paratyphi B</i> (1) <i>stanley</i> (1) <i>derby</i> (2) <i>bareilly</i> (2) <i>enteritidis</i> (1) <i>give</i> (1)
2 cats	<i>paratyphi B</i> (1) <i>typhimurium</i> (1)
4 horses	<i>inganda</i> (1) <i>bovis-morbificans</i> (2) <i>anatum</i> (1)
3 rabbits	<i>bareilly</i> (2) <i>houten</i> (1)
8 guinea-pigs	<i>stanley</i> (1) <i>heidelberg</i> (1) <i>augustenburg</i> (1) <i>kentucky</i> (1) <i>weltevreden</i> (3) <i>mgulani</i> (1)

The 10 most common serotypes (Table 6) accounted for 82.2% of all isolations, with the remaining 73 serotypes comprising 17.8%. The most frequently isolated serotype was *S. dublin*, accounting for 37.4% of all isolates. Twenty-six serotypes were isolated only once during the 5-year period. *S. typhimurium* had the widest zoological distribution; *S. pullorum-gallinarum* was not isolated during the period.

#### DISCUSSION

The 2322 salmonella serotypes presumably included all isolations made from animals and other non-human sources in Peninsular Malaysia. Under the Animal Salmonella Surveillance Programme, all veterinary laboratories in Peninsular Malaysia are required to submit all salmonella isolates to the VRI. It is possible that isolations of salmonella from animal and non-human sources may have been made by institutions outside the Department of Veterinary Services, although in the past such isolates were also sent to the VRI for confirmation and serotyping.

The 1981–5 surveillance period registered a 356% increase over the previous 5-year period (Joseph *et al.* 1986). This steep increase was largely due to the continuation of the *S. dublin* outbreak in cattle, which peaked in 1982, and the occurrence of new outbreaks of *S. typhimurium* and *S. paratyphi B* in cattle (Joseph, 1986) and *S. kentucky* in chickens. These serotypes caused severe morbidity and mortality in cattle (Joseph, 1986) and chickens. During the period, sampling of edible animal products, especially imported meats and frog legs, and the monitoring of the environment (hatcheries and poultry houses) were initiated. These activities resulted in a large number of isolations of salmonella.

The increase in bovine salmonellosis is striking. For the first time cattle became the major contributor to animal salmonellosis, accounting for 67.5% of the

Table 4. *Salmonella* isolations from wild and captive animals, 1981—5

No. and animal species	Source	<i>Salmonella</i> serotype (no. of isolations)
2 Java sparrows ( <i>Padda oryzivora</i> )	Pet shop	<i>typhimurium</i> (2)
2 budgerigars ( <i>Melopsittacus undulatus</i> )	Pet shop	<i>typhimurium</i> (1) <i>weltevreden</i> (1)
2 munias ( <i>Lonchura</i> sp.)	Zoo	<i>typhimurium</i> (2)
1 pigeon ( <i>Columbia livia</i> )	Pet shop	<i>typhimurium</i> (1)
1 parakeet ( <i>Psittacula</i> sp.)	Pet shop	<i>hvitlingfoss</i> (1)
1 parrot ( <i>Psittacus</i> sp.)	Pet shop	<i>typhimurium</i> (1)
2 toucans ( <i>Ramphastos</i> sp.)	Zoo	<i>toricada</i> (2)
1 collared scops-owl ( <i>Otus bakkamoena</i> )	Zoo	<i>blockley</i> (1)
1 crow pheasant ( <i>Lophura</i> sp.)	Zoo	<i>paratyphi B</i> var. <i>java</i> (1)
1 common peafowl ( <i>Pavo cristatus</i> )	Zoo	<i>matopeni</i> (1)
2 jungle fowls ( <i>Gallus gallus</i> )	Zoo	<i>hindmarsh</i> (2)
2 quails ( <i>Coturnix coturnix</i> )	Farms	<i>stanley</i> (1) <i>lexington</i> (1)
1 banded leaf monkey ( <i>Presbytis</i> sp.)	Zoo	<i>paratyphi B</i> var. <i>java</i> (1)
3 Muller's gibbons ( <i>Hylobates mulleris</i> )	Zoo	<i>hindmarsh</i> (1)
1 Sumatran deer ( <i>Cervus unicolor equinus</i> )	Farm	<i>paratyphi B</i> (1)
1 mouse deer ( <i>Tragulus javanicus</i> )	Zoo	<i>typhimurium</i> var. <i>copenhagen</i> (1)
1 puma ( <i>Felis concolor</i> )	Zoo	<i>blockley</i> (1)
8 house rats ( <i>Rattus rattus diardii</i> )	House	<i>weltevreden</i> (8)
2 pythons ( <i>Python reticulatus</i> )	Zoo	<i>lohbruegge</i> (2)

Table 5. *Salmonella* isolations from environment and feed samples, 1981—5

Source	No. of isolations	No. of serotypes
Hatcheries	78	16
Chicken houses	36	9
Cattle farm pond	3	1
Animal feeds	5	4
Total	122	19*

\* Some serotypes were recorded from more than one source.

isolates from non-human sources. The pattern of animal salmonellosis changed further during this 5-year period because of the reduced incidence of salmonella isolations from avian species. This has resulted in a reversal of the avian to mammalian salmonella ratio from 1.5:1 in the 1976–80 period (Joseph *et al.* 1986) to 0.2:1 for the current period. The total absence of *S. pullorum-gallinarum* isolations during this period is one of the significant highlights. It used to be the most frequently isolated serotype in all the previous surveillance periods. This apparent eradication of pullorum disease from Peninsular Malaysia is a result of the successful implementation of the National Pullorum Disease Eradication Programme. The Programme was revised in 1984 to make hatchery and poultry-house monitoring the basis of certification.

Wildlife transmission of salmonella infections is well recognized, and the literature abounds with isolations of salmonellae from exotic and bizarre hosts.

Table 6. *The ten most frequently reported salmonella serotypes from human and non-human sources in Malaysia, 1981-5*

Human*			Non-human				
Rank	Serotype	No.	%	Rank	Serotype	No.	%
1	<i>typhi</i>	3251	37.1	1	<i>dublin</i>	868	37.4
2	<i>weltevreden</i>	970	11.1	2	<i>typhimurium</i>	502	21.6
3	<i>blockley</i>	744	8.5	3	<i>sofia</i>	174	5.8
4	<i>stanley</i>	424	4.8	4	<i>weltevreden</i>	97	4.2
5	<i>paratyphi B</i>	332	3.8	5	<i>kentucky</i>	91	3.9
6	<i>lexington</i>	324	3.7	6	<i>blockley</i>	62	2.7
7	<i>bareilly</i>	304	3.5	7	<i>javiana</i>	45	1.9
8	<i>typhimurium</i>	303	3.5	8	<i>emek</i>	43	1.9
9	<i>hadar</i>	263	3.0	9	<i>choleraesuis</i> var. <i>kunzendorf</i>	34	1.5
10	<i>agona</i>	228	2.6	10	<i>paratyphi B</i> var. <i>java</i>	32	1.4
Total for top 10		7143	81.6	Total for top 10		1908	82.2
Total for all		8755		Total for all		2322	
No. of serotypes		88		No. of serotypes		83	

\* Source: Annual Reports of the Institute for Medical Research, Kuala Lumpur, Malaysia for the years 1981 to 1985.

During this surveillance period there were 35 isolations, a drop of 20 from the last period (Joseph *et al.* 1986). However, in the preceding period the majority of the wildlife isolations were made from one species, namely the house shrew (Joseph, Ham Thong Yee & Sivanandan, 1984). During this surveillance period isolations were made from 19 different wild and captive birds, mammals and reptiles. Most of these cases were from dead animals in zoos and pet shops, where salmonella may have been introduced through contaminated feeds.

*S. typhimurium* and *S. weltevreden* had the widest zoological distribution. These two serotypes are among the top ten salmonella serotypes isolated during the period (Table 6) from both human and non-human sources, and are important causes of morbidity in man and animals as well as mortality in the latter. The total of 83 serotypes recorded during this 5-year period is an increase of 27 over the previous 5-year period. With 34 new serotypes recorded, the total number of serotypes recorded from non-human sources in Peninsular Malaysia has increased from 63 (Joseph *et al.* 1986) to 97. Twenty-six of these serotypes were isolated only once during this 5-year period and are of little epidemiological significance. A third of the new serotypes recorded for the first time were the result of contaminated imported frozen meat (of cattle and buffaloes) and frogs' legs. This is an area of concern, as some of these new serotypes (*S. isangi*, *S. hadar*, *S. nchanga*, *S. mgulani*) have been isolated from man in this country during the same period (Annual Reports, 1981-5).

Jegathesan (1984) records a total of 104 serotypes from man in Malaysia for the period 1973-82. From 1983 to 1985, three more serotypes were isolated from man (Annual Reports 1981-5), to bring the total to 107. About 80 of the 97 serotypes so far isolated from non-human sources have also been isolated from man in this



country. One of the notable exceptions is *S. dublin*. Although it was reported as having occurred in man (Jegathesan, 1984) it was subsequently ascertained that they were animal isolates (Jegathesan, personal communication). This may be significant, as *S. dublin* was the most frequently isolated serotype from animals. The measures taken for the control of *S. dublin* infection in cattle farms appear to have been successful in limiting the infection to cattle (Joseph, 1986) because cattle handlers and milkers at the infected farms were faecal-screened on two occasions and found negative for *S. dublin*. However, the degree of significance of the control measures is difficult to assess because *S. dublin* is host-adapted to cattle and is rarely isolated from humans.

The public health importance of animal salmonellosis is well known. Control of salmonellosis in animals and salmonella contamination of edible animal products will, therefore, reduce the incidence of salmonella (other than *S. typhi*) infection in man.

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