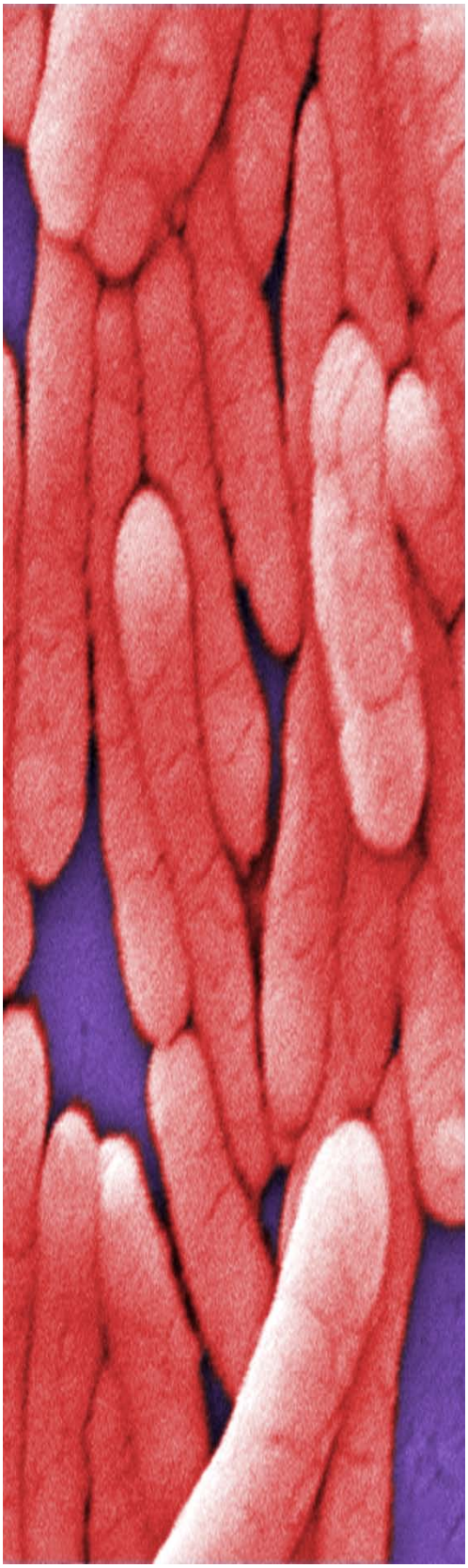
National Enteric Disease Surveillance: *Salmonella* Surveillance Overview



Surveillance System Overview: National Salmonella Surveillance

Salmonella is estimated to cause more than 1.2 million illnesses each year in the United States, with more than 23,000 hospitalizations and 450 deaths (1). *Salmonella* infections most often cause gastroenteritis which can range from mild to severe; invasive infections can be severe and potentially life threatening.

National *Salmonella* surveillance data are collected through passive surveillance of laboratory-confirmed human *Salmonella* isolates. Clinical diagnostic laboratories submit *Salmonella* isolates to state and territorial public health laboratories, where they are confirmed and serotyped according to the Kauffmann-White scheme. Unusual or untypable serotypes are forwarded to the Centers for Disease Control and Prevention's (CDC) National *Salmonella* Reference Laboratory at the Enteric Diseases Laboratory Branch (EDLB) for further characterization or confirmation; results are reported back to state and territorial public health laboratories. State and territorial public health laboratories send reports electronically to CDC through a variety of mechanisms. Initially, all surveillance data were transmitted through the Public Health Laboratory Information System (PHLIS), but other methods of data transmission have been implemented over time; currently data are collected into the Laboratory-based Enteric Disease Surveillance (LEDS) system, which has replaced PHLIS. The Division of Foodborne, Waterborne, and Environmental Diseases (DFWED) in the National Center for Emerging and Zoonotic Infectious Diseases maintains the national *Salmonella* surveillance data in LEDS. The annual summaries of these data are the only regularly published national source of serotype information for *Salmonella* (2).

Isolates are reported by state and represent the state where laboratory confirmation and subtyping occurred; the reporting state may not be the same as the state of residence of the person from whom the isolate was obtained. Reports include basic demographic information, serotype, and specimen source. For *Salmonella* serotype Typhi, only the first isolation in a calendar year for each person is counted. For serotypes other than Typhi, only the first isolation within a thirty day period for each person is counted, if the serotype and clinical source (e.g., stool or blood) are the same.

Rates of isolation are reported, but the rates of incomplete and unknown serotype data vary by state and year, as do reporting rates. The national *Salmonella* surveillance data are dynamic; data from previous years may change as isolate reports are added or corrected.

Salmonella isolates from animals and related sources (e.g., environment and feeds) are submitted by animal disease diagnostic laboratories and United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) laboratories throughout the United States to the Animal and Plant Health Inspection Services, National Veterinary Services Laboratories (USDA/APHIS/NVSL) for serotyping (3). *Salmonella* serotype data from animals and related sources that NVSL receives from other US laboratories are also included. Clinical animal (referred to as "clinical/non-human") isolates are defined as *Salmonella* isolates from animals with clinical signs of salmonellosis; *Salmonella* isolates identified through herd and flock monitoring and surveillance, feed sample testing, environmental testing, and USDA FSIS food testing programs are designated "non-clinical/non-human" isolates. Samples originating from non-human sources are tested for *Salmonella* for a variety of purposes and are obtained in multiple ways. Sampling is therefore neither complete nor representative, and any interpretation of these data should consider these limitations.

Although all *Salmonella* infections are nationally notifiable, for several reasons many cases are likely not recognized. Not all persons ill with *Salmonella* infection seek medical care, healthcare providers may not obtain a specimen for laboratory diagnosis, or the clinical diagnostic laboratory may not perform the necessary diagnostic tests. Additionally, not all *Salmonella* isolates may be forwarded or reported to state public health laboratories and are therefore not reported to national-level surveillance (4).

Other sources of national-level *Salmonella* surveillance data

Several other systems at CDC conduct surveillance for *Salmonella* infection. The National Notifiable Diseases System (NNDSS) collects and compiles reports of nationally notifiable infectious diseases, including salmonellosis (5). NNDSS collects data from states on both laboratory-confirmed and probable cases of infection (probable cases are defined as clinically ill persons with an epidemiological link to a confirmed case). NNDSS data is collected from states in a number of mechanisms, including the National Electronic Diseases Surveillance System (NEDSS) which is being developed to integrate both epidemiologic and laboratory information; currently laboratory information is not available from NNDSS. The National Antimicrobial Resistance Monitoring System (NARMS) monitors antimicrobial resistance among enteric bacteria (including *Salmonella*) from humans, retail meats, and food animals (6). The National Outbreak Reporting System (NORS) collects reports of foodborne, waterborne, enteric person-to-person, and animal contact-associated disease outbreaks from state and territorial public health agencies (7).

Overview of *Salmonella* Taxonomy and Serotype Nomenclature

I. Salmonella *Taxonomy*

The genus *Salmonella* is divided into two species, *enterica* and *bongori*. The species *Salmonella enterica* is further subdivided into six subspecies that are designated by taxonomic names; these are sometimes abbreviated by Roman numerals. The Roman numeral designations are used in designating serotypes by formula.

Salmonella enterica subspecies	
I	*Salmonella enterica* subsp. *enterica*
II	*Salmonella enterica* subsp. *salamae*
IIIa	*Salmonella enterica* subsp. *arizonae*
IIIb	*Salmonella enterica* subsp. *diarizonae*
IV	*Salmonella enterica* subsp. *houtenae*
VI	*Salmonella enterica* subsp. *indica*

Subspecies IIIa and IIIb were originally considered a separate genus, *Arizonae*, and are still sometimes referred to by this name, although it is obsolete. Despite this common history, subspecies IIIb is more closely related to the other *Salmonella enterica* subspecies than to subspecies IIIa.

Salmonella bongori was originally designated *S. enterica* subspecies V; it has since been determined to be a separate species of *Salmonella*. However, for simplicity and convenience, these strains are still sometimes referred to as "subspecies V".

II. Salmonella *Serotypes*

Serotyping is a subtyping method used to differentiate isolates of *Salmonella* beyond the subspecies level. *Salmonella* serotypes are designated based on the immunoreactivity of two cell surface structures, the O and H antigens. A substantial amount of diversity exists in these two antigens, resulting in the designation of more than 2,500 serotypes and the regular recognition of new serotypes.

There are national *Salmonella* surveillance data by serotype going back to 1963. Serotyping is an essential component of epidemiological surveillance and investigation of outbreaks of salmonellosis. Pulsed-field gel electrophoresis (PFGE) pattern characterization provides further subtyping. Historically, serotypes were considered different species (e.g., *Salmonella enterica* serotype Typhimurium was originally designated *Salmonella typhimurium*). It is now known that different serotypes of *Salmonella* can be closely related both phenotypically and genetically; serotypes are not intended as taxonomic designations.

IIa. *Salmonella* Serotype Antigens

The O antigen is a carbohydrate (polysaccharide) antigen that is the outermost component of the cell surface lipopolysaccharide. It is a polymer of O subunits and is typically composed of four to six sugars. Differences between O antigens can result from:

- the sugar components of the O subunit,
- the nature of the covalent bond between the sugars within the O subunit, or
- the nature of the linkage between the O subunits that form the O antigen polymer.

Specific epitopes within O antigens are divided into two categories: O group antigens and ancillary O antigens. O group antigens are associated with the core sugar configuration of the O antigen structure; O groups are designated by the primary O epitopes that are associated with the group. Ancillary O antigens are additional carbohydrates that are added to the core O antigen structure. They are associated with specific O serogroups and are often variably present or variably expressed.

Each O epitope is designated by a number; however, many of the common O groups were originally designated by letter and are still commonly referred to this way (e.g., serotype Typhimurium belongs to Group O:4 or Group B, serotype Enteritidis belongs to group O:9 or Group D1; serotype Paratyphi A belongs to Group O:2 or Group A). When multiple O epitopes are present, they are listed sequentially and separated by commas. Table A lists the 46 described O groups and the ancillary O antigens that may be present in serotypes of that group.

The H antigen is the filamentous portion of the bacterial flagellum; it is made up of protein subunits called flagellin. The C′ and N′ termini of flagellin, which give the flagellum its characteristic filamentous structure, are conserved. The antigenically variable portion of flagellin is the middle region, which is exposed on the surface of the flagellum. *Salmonella* is unique among enteric bacteria in that it can express two different flagellin antigens. The two antigens are referred to as Phase 1 and Phase 2. Typically, only one antigen is expressed at a time in a single bacterial cell. "Monophasic" isolates are those that express only a single flagellin type. These occur naturally for some serotypes (e.g., serotypes Enteritidis, Typhi, and most subspecies IIIa and IV serotypes) or can occur through the loss or lack of expression of a flagellin gene.

Table B lists the H antigens of *Salmonella*. Some antigens are composed of multiple factors, which are separated by commas in the formula; for example, the second phase antigen of serotype Typhimurium is composed of factors 1 and 2, which is represented as "1,2". Related antigens are grouped into complexes. For example, the 1 complex is composed of flagellar antigens 1,2; 1,5, 1,6 and 1,7.

IIb. *Salmonella* Serotype Identification

Salmonella serotypes are typically identified through a series of tests. Isolates are first identified to the genus and species level. The subspecies is then determined, typically by biochemical testing. O and H antigens are detected in independent agglutination assays using antisera that react with groups of related antigens or a single antigen. Both H antigens can sometimes be detected in a single culture, particularly for older strains or for isolates that have been passed multiple times. When only one H antigen is detected, the isolate is inoculated onto phase reversal

media, a semisolid media containing antisera to the H antigen that has already been identified. Organisms expressing the previously detected H antigen are immobilized by the antisera and grow only near the point of inoculation. Organisms expressing the second H antigen are able to move away from the point of inoculation, evidenced by growth throughout the media. The second H antigen is then determined using isolates from the phase reversal media.

IIc. *Salmonella* Serotype Designation

Salmonella serotypes are designated according to the conventions of the Kauffmann-White Scheme. The Kauffmann-White Scheme is maintained by the WHO Collaborating Centre for Reference and Research on *Salmonella* at the Institut Pasteur and is used by most public health laboratories worldwide (8). All *Salmonella* serotypes can be designated by a formula; subspecies I serotypes are also given a name (e.g., Typhimurium, Enteritidis, Typhi).

The typical format for a serotype formula is:

Subspecies [space] O antigens [colon] Phase 1 H antigen [colon] Phase 2 H antigen

Examples:

I 4,5,12:i:1,2 (*S. enterica* serotype Typhimurium)

I 4,12:i:1,2 (*S. enterica* serotype Typhimurium var. O:5-)

I 9,12:g,m:- (*S. enterica* serotype Enteritidis)

II 47:b:1,5 (*S. enterica* serotype II 47:b:1,5)

IV 48:g,z51:- (*S. enterica* serotype IV 48:g,z51:-)

IIIb 65:(k):z (*S. enterica serotype* IIIb 65:(k):z)

Other conventions:

- Some O and H epitopes are present variably. When the variable epitope is known to be encoded by a bacteriophage it is underlined (e.g., O20 is designated O:8,<u>20</u> in some serogroup C2 serotypes). Bacteriophage-encoded antigens have only been described for O antigens. When the basis for variability is not known, the antigenic factor is placed in square brackets (e.g., O5 is designated O:4,[5],12 in some serogroup B serotypes). For an individual isolate, if the variable factor is detected it is included in the formula without additional notation (i.e., without underlining or square brackets). If the variable factor is not detected, it is not listed in the formula.
- Some O and H factors are variably expressed. Weakly recognized antigens are indicated by parentheses (e.g., O antigen (6),14 or H antigen (k)).
- "Serotype" and "serovar" are used interchangeably.
- In monophasic isolates, the absence of an H antigen is indicated by a minus sign ("-") for the particular phase.
- Variants of serotypes that do not express all the recognized antigens characteristic of that particular serotype are not uncommon. This is a particular issue for subspecies I serotypes, because a serotype name cannot be assigned unless all the

antigens specified in the Kauffmann-White scheme for that serotype are identified. Isolates missing one or more antigens are designated by a formula. For example:

a) Monophasic variants are variants of serotypes that are typically diphasic; they lack the expression of either the flagellar Phase 1 or Phase 2 antigen. These are indicated by a minus sign ("-") in place of the missing phase (e.g., monophasic variants of serotype Typhimurium lacking the second phase H antigen, 1,2, are designated as *Salmonella* serotype I 4,5,12:i:- or I 4,12:i:-; monophasic variants of serotype Typhimurium that lack the first phase H antigen, i, are designated as serotype I 4,5,12:-:1,2 or I 4,12:-:1,2).
b) Nonmotile variants express no H antigens and are indicated by minus signs in both phases or by "nonmotile" in place of the H antigens (e.g., serotype I 4,5,12:nonmotile or I 4,5,12:-:-)
c) Rough variants are isolates that do not express O antigen. This is indicated by "Rough" in place of the O antigen in the antigenic formula (e.g., serotype I Rough:i:1,2).
d) Mucoid variants express a capsule that prevents immunologic detection of the O antigen. They are indicated by "Mucoid" in place of the O antigen in the antigenic formula (e.g., serotype I Mucoid:i:1,2).

- Rarely, isolates express a third H antigen that is noted by a colon followed by the antigen after the Phase 2 H antigen (e.g., serotype II 9,12:g,m,[s],t:1,5,7:z42, in which antigen z42 is the third H antigen).

All serotype information should be submitted to LEDS, whether or not a serotype "name" can be assigned to a strain. Monophasic, nonmotile, rough, and mucoid strains should be reported by formula indicating the antigens that were detected, as described above

III. Modified Kauffmann-White Scheme

CDC used a modified Kauffmann-White Scheme through 2002, and then changed to the Kauffmann-White Scheme to improve the comparability of United States *Salmonella* surveillance data with data from other countries. The primary differences between the two schemes are the following:

- Under the Kauffmann-White Scheme, subspecies I serotypes are named; subspecies II through VI serotypes are identified by formula. The Modified Kauffmann-White Scheme used names for subspecies II through VI serotypes through 1968 and formulas for subspecies II through VI serotypes after 1968. The most common serotypes that do not belong to subspecies I and were affected by the change to the Kauffman-White Scheme are
  a) Serotype Marina (now designated as IV 48:g,z51:-)
  b) Serotype Flint (now designated as IV 50:z4,z23:-)
  c) Serotype Kralendyk (now designated as IV 6,7:z4,z24:-)
  d) Serotype Chameleon (now designated as IV 16:z4,z32:-)

- Under the Kauffmann-White Scheme, serogroups E2 and E3 were combined with serogroup E1, because the antigenic changes in serogroups E2 and E3 are the result

of lysogenic conversion by bacteriophages and thus represent minor variants of serogroup E1 serotypes, designated as "variety" or "var.". The Modified Kauffmann-White Scheme used separate serotype names for these variants. Two common serotypes were affected by the merging of serogroups E2 and E3 with serogroup E1:

a) Serotype Newington (now Anatum var. 15+
b) Serotype Newbrunswick (now Give var. 15+)

- Under the Kauffmann-White Scheme, two biotypes of serotype Paratyphi B are recognized; they are differentiated primarily by the ability to ferment tartrate. Serotype Paratyphi B is tartrate-negative (unable to ferment tartrate) and is associated with severe, typhoid fever-like disease. Serotype Paratyphi B var. L(+)tartrate+ (formerly Java) is tartrate-positive (able to ferment tartrate) and is commonly associated with gastroenteritis. The two biovars of Paratyphi B can be confused because they have the same antigenic formula (I 4,[5],12:b:1,2), and are typically differentiated only by the ability to ferment tartrate, although PCRs that detect specific virulence markers are becoming more common. Given the very different disease syndromes caused by these two biotypes, it is important to accurately identify and report the two biotypes.

After the 2004 *Salmonella* Surveillance Summary was published, serotype designations for many isolates that were submitted during 1995 through 2003 were updated in the national *Salmonella* surveillance database using additional information submitted to CDC by states; previous surveillance summaries were not updated to reflect these change. CDC now uses all information submitted with the isolate, including information in the comments field, to characterize isolates more completely. This has affected the national *Salmonella* database in the following ways:

- Reporting of *Salmonella* serotype I 4,[5],12:i:- was inconsistent in the past due to variability in the nomenclature used to report this serotype, resulting in many isolates being reported only as "Group B" or "Subspecies I" and some isolates being incorrectly reported as serotype Typhimurium;
- Most variants of serotypes (monophasic, nonmotile or rough isolates) were not listed by their variant formulas before 2002, but were reported only by O group or subspecies. Since 2002, all serotype variants have been converted to standard serotype formulas whenever possible and incorporated into the surveillance database and reports;
- Serotypes of subspecies other than I were not listed in CDC surveillance summaries before 2002; instead, these isolates were reported by O group or subspecies only. Beginning in 2002, all serotype formulas that were submitted to the national surveillance system, regardless of subspecies, have been incorporated into the surveillance database and reported in surveillance summaries;
- In 2002, CDC modified the designation and reporting of partial serotype data. Before 2002, partially serotyped isolates were reported primarily by serogroup. Serogroups A–E are primarily composed of subspecies I serotypes, but most other

serogroups (F through Z) include serotypes from more than one subspecies. Therefore, when full serotype information is not available, isolates are reported first by subspecies, then O group and any additional serotype antigens.

IV. Abbreviating Salmonella *Serotype Designations*

As described above, the complete, formal designation of a *Salmonella* serotype is its genus-species or genus-species-subspecies name, followed by "serotype" and the serotype name or formula.

Some examples are:

Salmonella enterica subspecies *enterica* serotype Typhimurium
Salmonella enterica serotype Typhimurium
S. enterica serotype Typhimurium
S. enterica subspecies *salamae* serotype 47:b:1,5
S. enterica serotype II 47:b:1,5

Some scientific journals require the formal designation of serotypes throughout a paper; others allow the use of an abbreviation. However, there is no international standard for abbreviating *Salmonella* serotypes.

Because in the past, serotype names were written as species, and because inconsistencies in nomenclature still occur, it is helpful to include the word "serotype", which can be abbreviated "ser.", in a serotype abbreviation.

Examples of clear abbreviations are:

Salmonella serotype Typhimurium
Salmonella ser. Typhimurium
Salmonella serotypes Typhimurium, Enteritidis, and Newport

A commonly used but undesirable abbreviation designates the genus- name as "*S.*", omits the species name, and is followed by the serotype name (e.g., *S.* Typhimurium).

This format has the following disadvantages:

- It can be misinterpreted as giving species/taxonomic standing to serotypes.
- It is formatted as a taxonomic designation. However, serotypes have no taxonomic standing, and this abbreviation is not correct taxonomically. To be taxonomically correct, the name must include species (e.g., *S. enterica* ser. Typhimurium).
- "*S.* " could mean *Shigella*, *Serratia, Sutterella*, or any of a number of genera. This is most likely to be a problem with less common serotypes or when a variety of genera are being considered.
- "*S.* Cholerasuis" and "*S.* Enteritidis" were historically used as taxonomic species names. The incorrect abbreviations "*S.* Cholerasuis" and "*S.* Enteritidis", which are intended to denote serotypes, are difficult to differentiate from the historical species names, which are sometimes still used and found in the literature.

V. Salmonella *Serotype Statistics*

As of 2007, 2,579 *Salmonella* serotypes had been described; about 60% belong to subspecies I. In the United States, 99% of reported human *Salmonella* isolates belong to subspecies I. The 20 most common serotypes from human specimens account for about 70% of all isolates reported in the United States; the top 100 serotypes account for about 98% of all isolates. In 2007, subspecies other than I were among the 100 most commonly isolated serotypes. These included two subspecies IV serotypes (IV 50:z4,z23:-; IV 48:g,z51:-;) and non-serotyped subspecies II, IIIa/IIIb, IIIb, and IV isolates. In general, subspecies IV isolates are the most commonly isolated subspecies other than I (particularly serotypes IV 48:g,z51:-; IV 50:z4,z23:-; IV 6,7:z4,z24:-; and IV 16:z4,z32-), followed by subspecies IIIb, II, and IIIa. Subspecies VI and S. bongori isolates are very rare.

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Table A. *Salmonella* O groups and associated O antigens

O Group (number designation)	O Group (letter designation	Antigens present in all serotypes	Additional antigens that may be present in some serotypes
2	A	2,12	1
4	B	4,12	1; 5; 27
7	C1	6,7	14; (Vi)
8	C2	8	6; 20
9	D1	9,12	1; (Vi)
9,46	D2	9,46	none
9,46,27	D3	9,12,46,27	1
3,10	E1	3,10	15; 15,34
1,3,19	E4	1,3,19	10; 15
11	F	11	none
13	G	13	1; 22; 23
6,14	H	6,14	1; 24; 25
16	I	16	none
17	J	17	none
18	K	18	6; 14
21	L	21	none
28	M	28	none
30	N	30	none
35	O	35	none
38	P	38	none
39	Q	39	none
40	R	40	1
41	S	41	none
42	T	42	1
43	U	43	none
44	V	44	1
45	W	45	none
47	X	47	1
48	Y	48	none
50	Z	50	none
51		51	1
52		52	none
53		53	1
54 (provisional)		54	21; 3; 3,15; 4,12; 8,20; 6,7
55		55	none
56		56	none
57		57	none
58		58	none
59		59	1
60		60	none
61		61	none
62		62	none
63		63	none
65		65	none
66		66	none
67		67	none

Table B. H (flagellar) antigens of *Salmonella*

1 complex:	1,2	Other antigens (not part of a complex):	a
	1,5		b
	1,6		c
	1,7		d
	1,2,5		e,h
	1,2,7		i
	1,5,7		k
	1,6,7		(k)
EN complex:	e,n,x		r
	e,n,x,z15		r,i
	e,n,z15		y
G complex:	f,g		z
	f,g,m,t		z6
	f,g,s		z10
	f,g,t		z29
	g,m		z35
	g,m,p,s		z36
	g,m,q		z36,z38
	g,m,s		z38
	g,m,s,t		z39
	g,m,t		z41
	g,p		z42
	g,p,s		z44
	g,p,u		z47
	g,q		z50
	g,s,q		z52
	g,s,t		z53
	g,t		z54
	g,z51		z55
	g,z62		z56
	g,z63		z57
	g,z85		z60
	m,p,t,u		z61
	m,t		z64
L complex:	l,v		z65
	l,w		z67
	l,z13		z68
	l,z13,z28		z69
	l,z28		z71
Z4 complex:	z4,z23		z81
	z4,z23,z32		z83
	z4,z24		z87
	z4,z32		z88

Report compiled by:
Richard Bishop[1], Matthew M. Erdman, DVM, Ph.D.[2], Patricia Fields, Ph.D.[3], Katie Fullerton, MPH[4], Kelly Jackson, MPH[4], Barbara Mahon, MD, MPH[4]

[1]Biostatistics and Information Management Office, Division of Foodborne, Waterborne, and Environmental Diseases (DFWED), National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Office of Infectious Diseases (OID), Centers for Disease Control and Prevention (CDC); [2]Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories, United States Department of Agriculture; [3]Enteric Diseases Laboratory Branch, DFWED/NCEZID/OID/CDC, [4]Enteric Diseases Epidemiology Branch, DFWED/NCEZID/OID/CDC

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Centers for Disease Control and Prevention
Division of Foodborne, Waterborne and Environmental Diseases
Mail Stop D-63
1600 Clifton Rd
Atlanta, Georgia 30333
Telephone: 404.639.2206
http://www.cdc.gov/ncezid/dfwed/